THE UNIVERSITY OF ALBERTA

SPONTANEOUS MUTABILITY IN STRAINS OF *SACCHAROMYCES CEREVISIAE* SENSITIVE TO ULTRAVIOLET RADIATION

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Spontaneous Mutability in Strains of Saccharomyces cerevisiae Sensitive to Ultraviolet Radiation" submitted by Teresa Brychcy in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

In order to correlate DNA repair mechanisms with those of spontaneous mutation in the yeast Saccharomyces cerevisiae, the spontaneous reversion rates to lysine and histidine independence of several UV-sensitive strains were measured by means of the 1000-compartment fluctuation test. The lys1-1 allele is a nonsense-suppressible mutant and is revertible, either by a reversion at the lys1 locus itself, or by a forward mutation at one of eight supersuppressor loci. The his1-7 allele is believed to be a missense mutant and can be reverted by internal missense suppression.

Of the 22 gene loci controlling sensitivity to ultraviolet light in Saccharomyces cerevisiae, nine radiosensitivity alleles were studied for their effects on spontaneous mutability. The excision-defective allele rad3-12 and the alleles rad5-1 and rad18-2 were found to significantly increase the spontaneous rate of reversion to lysine independence in strains bearing them. The alleles rad3-12 and rad5-1 also increased the histidine reversion rate. Strains bearing the other excision-defective alleles tested, rad1-1, rad2-2, and rad4-3, and the rad10-1 allele, however, failed to show any significant increase in spontaneous mutability, although the rad1-1 allele displayed a certain amount of variability in different genetic backgrounds. The effects of rad8-1 and rad18-1 could not be determined and were attributed to modifiers, present in the genetic background, which exert their effects on the spontaneous mutability of these strains. The results obtained both by qualitative as well as quantitative measurements are in good agreement.

On this basis, it is suggested that the increased spontaneous mutation rate and UV-sensitivity in strains bearing the rad5-1 allele are likely to be due either to the same mutation, or else, to independent mutations in two very closely linked genes. Similar conclusions might be applicable to rad3-12 and rad18-2, though the data for these alleles are sparser. Thus, it is concluded that at least some genes controlling sensitivity to ultraviolet light may affect spontaneous mutability in yeast. An interesting feature of the three UV-sensitive mutators described herein is that their wild type gene products are believed to mediate the first step in each of three pathways leading to repair of UV-induced DNA damage (Game and Cox, 1973; Cox and Game, 1974).

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INTRODUCTION

The problem of how spontaneous mutations arise remains a perplexing one to this day. Many and varied theories have been suggested to explain spontaneous mutability (see Magni, 1969 for review). Among these, errors in DNA replication, errors in the repair of damaged DNA, and mistakes in genetic recombination have been cited as the principal sources of spontaneous mutations (von Borstel, 1968, 1969a).

It is now known that radiation-induced DNA damage can be repaired through several different mechanisms. Also, mistakes in the repair of such damage have been postulated to lead to an increase in the frequency of radiation-induced mutations. An important question which arises is whether spontaneously arising premutational defects in the DNA can actually be removed by the same mechanisms which repair radiation-induced damage. In other words, do errors in the repair of radiation-induced DNA lesions have any effect on spontaneous mutation rates? With the hope of finding a solution to the problem of the relation, if any, between repair and spontaneous mutability, studies of spontaneous mutation rates in mutants sensitive to radiation have been undertaken in recent years.

Causes of Radiation-Sensitivity

The isolation in 1958 by Hill of a mutant of the bacterium Escherichia coli B which was sensitive to ultraviolet light and

X-rays initiated the study of radiation-sensitivity in prokaryotes and eukaryotes. Since then, radiation-sensitive strains have been discovered in a variety of organisms, and it has become apparent that sensitivity to both ionizing and non-ionizing radiation is under genetic control.

Radiation-sensitive strains are presumed to lack completely or at least have a decreased ability to repair radiation-induced DNA damage (Witkin, 1966), which, in the case of ultraviolet light, consists mainly of pyrimidine dimers, the most frequently produced being those of thymine (Setlow et al., 1963; Setlow, 1966; Strauss, 1968). Formed by the dimerization of two adjacent pyrimidines on the same DNA chain by linkage of the 5,6 unsaturated bonds to form a cyclobutane ring, pyrimidine dimers distort the phosphodiester backbone of the double helix (see Strauss, 1968 for review). The major overall effect on the cell of unrepaired pyrimidine dimers is to reduce the rate of DNA synthesis (Setlow et al., 1963; Bollum and Setlow, 1963), which in turn is lethal (Wacker et al., 1962). A given dose of ultraviolet radiation produces an equal number of dimers in both sensitive and insensitive strains of E.coli (Setlow et al., 1963). Whether or not a strain survives after irradiation depends on whether or not the radiation-induced defects are repaired (Haynes, 1966; Brendel and Haynes, 1973). Thus, mutations which increase sensitivity to UV may do so either by decreasing the ability of a strain to repair pyrimidine dimers or by decreasing its ability to tolerate unrepaired dimers and consequently form colonies (Witkin, 1969a).

Repair Mechanisms in E.coli

There exist three different types of mechanisms to repair ultraviolet light-induced DNA damage in radiation-insensitive strains of bacteria; namely photoreactivation, excision repair, and post-replication repair.

1. Photoreactivation. If an irradiated strain of bacteria is exposed to visible light, its photoreactivating enzyme is released from the irradiated DNA, to which it binds in the dark, and splits or monomerizes the pyrimidine dimers in situ (Wulff and Rupert, 1962; Cook, 1967), thus restoring normal DNA structure. Photoreactivation is specific for pyrimidine dimers only (Setlow, 1966) and both UV-killing and UV-induced mutation can be photoreactivated (see Witkin, 1969a for review). Survival in some radiation-sensitive bacteria can be increased by post-UV exposure to photoreactivating light (Setlow and Carrier, 1964). Thus the presence or absence of pyrimidine dimers can be quantitated by determination of the amount of photoreactivable damage.

2. Excision Repair. Because photoreactivable damage (pyrimidine dimers) can be reduced by dark storage in non-nutrient medium (liquid holding recovery) in bacteria (Roberts and Aldous, 1949), this indicates the existence of a dark repair process. The mechanism for such dark repair involves an enzyme or enzyme system which recognizes the double helix distortion caused by the dimer and introduces a single-stranded nick into the DNA strand near the dimer site. The damaged bases and a few adjacent nucleotides are then removed as oligonucleotides by the 5' exonuclease of the DNA polymerase I (Kelly et al., 1969). The gaps left in the DNA after excision are then

refilled by repair synthesis, carried out by a polymerase enzyme using the undamaged DNA strand as template. Evidence for dimer excision in E.coli comes mainly from observations of the disappearance of dimers from the acid insoluble fraction of UV-irradiated wild type cells, which have been dark incubated, and their appearance in the soluble fractions (Setlow and Carrier, 1964; Boyce and Howard-Flanders, 1964). No comparable dimer loss is however observed in UV-sensitive cells. The existence of non-conservative replication at random positions in the genome has been shown by density labelling experiments (Pettijohn and Hanawalt, 1964). The final step in excision repair involves the rejoining of the free end of the newly synthesized DNA to the old polynucleotide chain (McGrath and Williams, 1966) by means of a ligase enzyme (Mead, 1964; Olivera and Lehman, 1967).

3. Post-Replication Repair. A third, less well characterized mechanism for increasing survival after ultraviolet irradiation is believed to operate after DNA replication (Witkin, 1969a), as opposed to excision repair which functions prior to replication. Dimers not excised from the DNA before replication pass through the replication fork and are no longer subject to excision. Since they cannot be replicated, dimers give rise after the first post-UV DNA replication to gaps in the daughter DNA strands at positions opposite each dimer in the template (Howard-Flanders et al., 1968). These daughter strand gaps, however, gradually disappear during the hour following the first post-irradiation DNA replication, as is implied by the fact that low molecular weight irradiated DNA is eventually converted into high molecular weight DNA upon subsequent incubation (Rupp and

Howard-Flanders, 1968) in both wild type and excision-defective strains.

This repair mechanism is believed to involve recombination and may possibly be under the control of the recA gene in E.coli (Rupp et al., 1971). Recombination defective mutants show liquid holding recovery whereas double mutants defective in both recombination and excision repair show no liquid holding recovery (Howard-Flanders et al., 1969). Also, recA mutant DNA sediments as short molecules after post-UV incubation. The detection of molecules of intermediate density formed in UV-irradiated but not control bacteria in density labelling experiments indicates that recombinational repair may depend on sister duplex exchanges (Howard-Flanders et al., 1971; Rupp et al., 1971). The simplest mechanism envisualized for such repair is single-stranded exchange of a limited length at each dimer, such that daughter strand fragments are assembled into a complete genome and dimers remain in their original strands. Regions containing a dimer and a gap in one duplex will usually be intact in the sister duplex and thus will permit such exchanges (Howard-Flanders, 1968).

Error-Free Versus Error-Prone Repair Mechanisms

The concepts of error-free and error-prone repair have been put forward by (Witkin, 1969a) for UV-induced mutations in bacteria. Of the three DNA repair mechanisms, the least likely to introduce errors during repair is photoreactivation because it consists of a single enzymatic step. The process of dimer excision in bacteria is itself

also believed to be error-free. Rather, the unexcised dimers which pass through the replication fork seem to be responsible for mutation induction, as excision-defective strains of E.coli have a higher UV-induced mutation frequency than wild type strains and these induced mutations are photoreactivable (Witkin, 1966; Hill, 1965; Bridges et al., 1967). Thus, in contrast to photoreactivation and excision repair, the process of post-replication recombinational repair of daughter strand DNA gaps has been considered to be error-prone. While excision-defective bacteria display an increased mutability, recA mutant strains show no change in mutability, as expected if there is no error-prone recombinational repair (Witkin, 1969b). The presence of both the recA gene product (believed to be required for post-replication repair) and the exrA gene product (thought to decrease the accuracy of repair [Witkin, 1967]) seems to be necessary for this error-prone repair (Howard-Flanders, 1968). Based on studies with excision-defective, recombination-deficient, and polymerase-deficient strains, Kondo et al. (1970) have also concluded that, in addition to replication errors, recombination errors, arising from spontaneous or mutagen-induced premutational DNA damage, are responsible for base substitution mutations in E.coli.

Genes Controlling UV-Sensitivity in E.coli and Other Organisms

In Escherichia coli several loci are now known to have an effect on radiation-sensitivity (Howard-Flanders and Boyce, 1966; Strauss, 1968). The uvr genes control sensitivity to ultraviolet irradiation. The three genes uvrA, uvrB, and uvrC each determine a

step in pyrimidine dimer excision (Howard-Flanders et al., 1966). The rec loci when mutant decrease the efficiency of genetic recombination as well as affecting UV- and ionizing radiation-sensitivity (Clark and Margulies, 1965; Howard-Flanders and Theriot, 1966), but are normal in excision repair (Clark et al., 1966). A mutation at the lex locus (exr in E.coli B) affects UV and X-ray sensitivity but shows no appreciable recombination deficiency (Howard-Flanders and Boyce, 1966). Other loci which affect radiation-sensitivity include: lon mutants which have a decreased ability to divide after UV (Witkin, 1967); ras mutants which predominantly increase UV-sensitivity but can excise dimers and show normal genetic recombination (Walker, 1969); and pol mutants (De Lucia and Cairns, 1969) which have reduced levels of DNA polymerase I and show an increased UV-sensitivity, perhaps because they repair excision gaps at a very slow rate (Boyle et al., 1970; Witkin and George, 1973).

Genes affecting sensitivity to ultraviolet light have also been cited in Proteus mirabilis (Bohme, 1967), Micrococcus radiodurans (Hariharan and Cerutti, 1971), Chlamydomonas reinhardi (Davies, 1967), Aspergillus nidulans (Lanier and Tuveson, 1966; Fortuin, 1971), Neurospora crassa (Chang and Tuveson, 1967; Schroeder, 1970), Ustilago maydis (Holliday, 1965), Ustilago violacaea (Day and Day, 1970), Schizosaccharomyces pombe (Schupbach, 1971; Fabre, 1971), and Saccharomyces cerevisiae (see below).

UV-Sensitivity and Repair Mechanisms in Yeast

Mutants of the yeast Saccharomyces cerevisiae which are sensitive

to radiation have been isolated in recent years (Nakai and Matsumoto, 1967; Snow, 1967; Cox and Parry, 1968; Resnick, 1969; Zakharov et al., 1970; Averbeck et al., 1970; Lemontt, 1971a; Moustacchi, 1969). By means of complementation and recombination tests, these mutants have been assigned to 22 genetic loci which control sensitivity to UV (Game and Cox, 1971). Mutation at any one of these 22 rad loci confers a greater increase in sensitivity to ultraviolet light compared to the wild type. In addition, there are also eight known loci which affect sensitivity to X-rays (Game and Mortimer, 1974).

Similar repair mechanisms to those in bacteria are functional in wild type yeast. That a process of dark repair exists in yeast is evident from the increase in survival (liquid holding recovery) obtained by dark holding UV-irradiated wild type yeast in non-nutrient medium, such as distilled water or buffer, before plating (Patrick et al., 1964; Parry and Parry, 1972). That photoreactivation has no further effect on survival indicates that the pyrimidine dimers are removed by this process.

Excision repair cannot be directly demonstrated in yeast, as has been done in bacteria, because of the inability to introduce a specific label due partly to the absence of thymidine kinase (Cox and Parry, 1968; Grivell and Jackson, 1968). However, the effects of dark liquid holding are indicative of the occurrence of excision repair. For example, if dark holding causes no change or a decrease in survival and subsequent photoreactivation is able to increase the survival, then dimers (the photoreactivable damage) are not being properly excised and the strain is deficient in excision repair. The

effects of such post-treatments on certain UV-sensitive mutants indicated that rad1, rad2, rad3, and rad4 mutants cannot excise dimers or only poorly so (Parry and Parry, 1969). Similar effects leading to the same conclusion have been observed for all of the rad1 and rad3 alleles (Parry et al., 1972). Recently, by means of labelling yeast DNA with ^{14}C-uracil, it has been shown that rad1-1-bearing strains do not remove thymine dimers from their DNA after dark incubation, whereas a wild type strain loses them (Unrau et al., 1971). Thus the RAD1 wild type gene product is required for excision of UV-induced pyrimidine dimers. It has also been shown that UV-irradiated rad2-17 DNA extracts, which have been held in the dark,can compete with UV-irradiated Haemophilus influenzae DNA for photoreactivating enzyme while wild type extracts lose this ability (Resnick and Setlow, 1972). This may also be taken as evidence that the RAD2 product is involved in pyrimidine dimer excision. A comparison of their photoreactivability after UV, their photoreactivability after UV and dark holding, and their sensitivity to certain chemicals indicates a similarity between rad1-1 UV-sensitive yeast and excision defective (uvr) bacteria (Kilbey and Smith, 1969). Finally, if two gene loci control steps in the same repair pathway, the UV-sensitivity of the double mutant is no greater than that of one or the other of the single mutants (Game, 1971), an interaction defined as epistasis. Alleles of rad1, rad2, rad3, and rad4 have been shown to interact epistatically in double, triple, and quadruple mutants (Game, 1971; Game and Cox, 1972; Nakai and Matsumoto, 1967). This indicates that RAD1, RAD2, RAD3, and RAD4 genes control steps in the same repair

pathway, assumed, on the basis of the above evidence, to be that of excision repair.

A synergistic interaction, on the other hand, in which the sensitivity of the double mutant is greater than that expected from an additive interaction, indicates that the two loci involved mediate steps in different repair pathways (Game, 1971). As a result of a study of radiation-sensitive mutants displaying synergistic and epistatic interactions in different combinations, two more pathways, besides excision repair, have been devised for the dark recovery of yeast cells from pyrimidine dimer damage (Game and Cox, 1973; Cox and Game, 1974; Game, 1971). The first step in one of these is thought to be controlled by the RAD18 gene product which may be capable of repairing both UV- and X-ray-induced DNA damage, possibly through post-replication repair (Brendel and Haynes, 1973). Another pathway, under the control of the X-ray sensitive genes RAD51 and RAD50 may also repair UV-induced damage. Studies of the effects of dark holding and photoreactivation on UV-induced intragenic recombination support the possibility that RAD50 and RAD18 may be involved in dark recombination pathways (Hunnable and Cox, 1971).

The REV genes, which decrease UV-induced mutability, have also been suggested to perhaps be involved in post-replication repair (Lemontt, 1971b). One of rev mutants, rev2-1, is an allele of rad5. Since all photoreactivable dimer damage in rad5 mutants is removed by dark holding, this implies that the RAD5 gene is not involved in excision repair (Parry and Parry, 1969). Synergistic double mutant interactions (Lemontt, 1971a) indicate that the rev genes do not

block the same pathway as the rad2 and rad4 mutants.

In Saccharomyces cerevisiae post-replication repair may also be error-prone. The rev mutants, for example, since they decrease UV-induced revertibility, are thought to block a mutation-prone pathway (Lemontt, 1971b; Game, 1971). Some of the excision-defective rad alleles have an increased UV-induced mutability (Hunnable, 1972; Resnick, 1968). This implies that blocks to excision repair cause lesions to be repaired via an error-prone "escape" pathway, possibly the one controlled by the rev genes. In wild type yeast, a decrease in error-prone recombinational repair, with exclusively excision repair being functional, has been proposed as an explanation for the decrease in UV-induced mutations after liquid holding (Parry and Parry, 1972).

Spontaneous Mutability and UV-Sensitivity

If naturally occurring DNA defects which lead to spontaneous mutations are repaired by the same gene-controlled enzyme mechanisms which decrease radiation-induced DNA damage, then one might expect an increased spontaneous mutability in radiosensitive mutants in which repair is blocked (Hanawalt and Haynes, 1965). Or, at least some of the radiation-sensitive mutants, whose normal repair routes are blocked in such a way as to leave open only an error-prone pathway for repair, would show an increase in spontaneous mutability, that is, would be mutators.

Among the mutator strains of bacteria several are known to be associated with UV-sensitivity. High mutability and UV-sensitivity

were found to be inseparable by recombination in an E.coli K12 mutator strain (Mohn, 1968). Also in E.coli, the uvr502 mutant showed increased spontaneous mutability (Smirnov and Skavronskaya, 1971). The double mutant behavior of mutU4, allelic to uvr502 (Siegel and Kamel, 1974) indicates a possible function for the MUT gene product in excision repair (Siegel, 1973), such that mutU4 may repair such damage inaccurately or not at all. In Neisseria meningitidis, mutR1 was also found to confer increased UV-sensitivity and loss of UV-sensitivity led to loss of the mutator property (Jyssum, 1968). In Bacillus subtilis a UV-sensitive recombination-deficient strain showed an increased spontaneous reversion rate (Prozorov and Barabancikov, 1967). An increase in spontaneous mutability associated with increased UV-sensitivity was also found in Proteus mirabilis (Bohme, 1967). The observation that DNA polymerase mutants of E. coli show an increased frequency of spontaneous deletions (Coukell and Yanofsky, 1970) and are also UV-sensitive, implies that a component of the DNA repair system is affected. That excision repair and recombination repair are somehow involved in the repair of spontaneous DNA lesions in E.coli was concluded by Haefner (1968) because a deficiency of either type of repair enhances the frequency of spontaneous lethal sectoring.

Although evidence for the association of spontaneous mutability and UV-sensitivity exists, there also exists evidence showing that in some cases two different mutations can be responsible for these effects (Hill, 1968; Liberfarb and Bryson, 1970). Also, excision defective E.coli show normal spontaneous mutation rates to valine

resistance and arginine independence (Howard-Flanders and Boyce, 1966).

In Saccharomyces cerevisiae, a possible association of radiosensitivity with spontaneous mutability is also indicated. Increased spontaneous mutation rates to adenine independence in two UV-sensitive strains (one of which is rad2-18) have been found (Zakharov et al., 1968; Zakharov et al., 1970). Increased spontaneous reversion rates to histidine, adenine and leucine independence and to canavanine sensitivity were found in the uvs_2 (rad1-3) mutant (Moustacchi, 1969). One of the rev genes, rev2-1 (rad5-5) also showed a high average spontaneous mutation frequency to adenine independence (Lemontt, 1972). The rad18-2 mutant, which is both UV- and X-ray-sensitive showed an increased spontaneous reversion to lysine independence (von Borstel et al., 1971). The association of X-ray sensitivity with mutator activity (rad50 and rad52) has also been found (von Borstel et al., 1971; von Borstel et al., 1968).

Purpose of Study

Because some of the UV-sensitive mutants of Saccharomyces cerevisiae possess an increased spontaneous mutability, it would be of interest to determine accurately if any of the other UV-sensitive rad mutants of this yeast have a similar effect. Such a finding would perhaps aid in elucidating how radiation-sensitive mutants involved in different repair pathways might affect the rates of spontaneous mutation. The rad alleles studied were: rad1-1, rad2-2, rad3-12, rad4-3, rad5-1, rad8-1, rad10-1, rad18-1, and rad18-2. The

first four are alleles of each of the four rad loci involved in excision repair. The RAD5 and RAD18 loci may be involved in some other dark repair pathways, possibly a post-replication recombinational pathway. The functions of the RAD8 and RAD10 loci in repair are at the moment unknown, but they are probably not involved in excision as they show an increase in survival after dark holding and no change after photoreactivation (Parry and Parry, 1969).

MATERIALS AND METHODS

MATERIALS

Strains of Yeast

Radiation-sensitive strains of the yeast Saccharomyces cerevisiae were obtained from J.C. Game. Table 1 lists the haploid strains bearing the rad alleles used in this study, their genotypes, and the researchers who originally isolated them. The radiosensitivity alleles are designated both by their new interlab locus numbers (Game and Cox, 1971) as well as by their original designations. In Table 2 are given the haploid strains which do not carry a mutant radiosensitivity gene and therefore are insensitive to radiation. The strains X1687-12B, KC372, and KC376 were used as controls for ultraviolet light survival curve experiments. XV169-12A, XV169-15A, XV185-6A, and XV185-14C were used in crosses with the radiation-sensitive strains. The two latter and X464-1A, X464-20C, XV185-4A, and XV185-6D were used in complementation tests. The diploid strains obtained from crosses involving the radiation-sensitive strains are listed in Table 3. The genotypes of haploid strains obtained from these diploids and used in mutation rate studies are given in Tables 4 and 5.

In all tables, the symbols α and a represent complementary mating type alleles; trp, arg, his, lys, ade, hom, and leu imply the inability to grow in the absence of tryptophan, arginine, histidine, lysine, adenine, homoserine, and leucine, respectively; mal and gal

TABLE 1. Genotype and origin of haploid strains bearing the *rad* alleles used in this study

rad Allele (Interlab Locus Number)	Original Designation	Strain	Other Genotypic Markers	Origin
rad1-1	UV_1^S	XS774-5D	*a*	Nakai and Matsumoto (1967)
rad2-2	*uvs-8*	S226-7C	*α*	Snow (1967)
rad3-12	$uvs_{13\text{-}u8}$	197/2D	*α ade2-1 his*	Cox and Parry (1968)
rad4-3	*uvs-12*	S960-1A	*α*	Snow (1967)
rad5-1	*uvs-10*	S228-6B	*α*	Snow (1967)
rad8-1	$uvs_{8\text{-}u19}$	197/2D	*α ade2-1*	Cox and Parry (1968)
rad10-1	*uvs-14*	S962-3C	*α*	Snow (1967)
rad18-1	*uvs-18*	JCG133/3A	*α*	Snow (1967)
rad18-2	*uxs1-1*	KC372	*a arg4-17 his5-2 lys1-1 ade2-1 leu1-12*	Resnick (1969)
rad52-1	*xrs1-1*	KC376	*a arg4-17 his5-2 lys1-1 ade2-1 leu1-12*	Resnick (1969)

TABLE 2. Genotype and origin of haploid strains other than those bearing rad alleles

Strain	Genotype										Origin
XV169-12A	α	trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10				von Borstel (1973)
XV169-15A	a	trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10				von Borstel (1973)
XV185-4A	α	trp5-48	arg4-17	his5-2	lys1-1	ade2-1					von Borstel
XV185-6A	α	trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10				von Borstel (1973)
XV185-6D	a	trp5-48	arg4-17	his5-2	lys1-1	ade2-1					von Borstel
XV185-14C	a	trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10				von Borstel
X464-1A	a	trp1		his2		ade1		leu1	gal1	mal	Mortimer
X464-20C	α	trp1		his2		ade1		leu1	gal1	mal	Mortimer
X1687-12B	α	trp5-48	arg4-17	his5-2	lys1-1	ade2-1					Mortimer

TABLE 3. Origin of diploid strains

Diploid	Origin
XV185	X1687-12B XV169-15A
XV361	S226-7C rad2-2 XV169-15A
XV362	JCG133/3A rad18-1 XV169-15A
XV363	XS774-5D rad1-1 XV169-12A
XV365	S962-3C rad10-1 XV169-15A
XV366	XV365-3A XV169-15A
XV418	S960-1A rad4-3 XV185-14C
XV419	S228-6B rad5-1 XV185-14C
XV420	197/2D rad8-1 XV185-14C
XV421	XS774-5D rad1-1 XV185-6A
XV422	S226-7C rad2-2 XV185-14C
XV423	197/2D rad3-12 XV185-14C
XV424	XV362-5A XV185-14C

TABLE 4. Genotypes of auxotrophic haploid strains bearing a rad or its wild type allele involved in the excision repair pathway

Strain	Genotype							
XV363-1A	α	rad1-1		arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV363-2A	a	rad1-1		arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV363-10A	a	rad1-1		arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV363-11A	α			arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV421-5D	a		trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV421-12A	a	rad1-1	trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV421-15B	α		trp5-48		his1-7	lys1-1	ade2-1	hom3-10
XV421-17A	α	rad1-1	trp5-48		his1-7	lys1-1	ade2-1	hom3-10
XV361-1A	α	rad2-2	trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV361-2A	a		trp5-48		his1-7	lys1-1	ade2-1	hom3-10
XV422-1A	α	rad2-2		arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV422-2A	a	rad2-2	trp5-48		his1-7	lys1-1	ade2-1	hom3-10
XV422-3A	α		trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV422-4A	a			arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV423-1A	α	rad3-12			his1-7	lys1-1	ade2-1	hom3-10
XV423-2A	α	rad3-12	trp5-48		his1-7	lys1-1	ade2-1	hom3-10
XV423-3A	α			arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV423-4A	a			arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV418-1A	a	rad4-3	trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV418-2A	α	rad4-3		arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV418-3B	α		trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV418-8A	α		trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10

TABLE 5. Genotypes of auxotrophic haploid strains bearing a rad or its wild type allele involved in unknown repair pathways

Strain	Genotype							
XV419-2A	a	rad5-1	trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV419-5A	α	rad5-1			his1-7	lys1-1	ade2-1	hom3-10
XV419-11A	α			arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV419-12A	α				his1-7	lys1-1	ade2-1	hom3-10
XV419-13A	a	rad5-1		arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV420-1A	α	rad8-1	trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV420-2A	α	rad8-1		arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV420-3A	a	rad8-1		arg4-17		lys1-1	ade2-1	
XV420-4A	α		trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV420-6A	α		trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV365-2A	a	rad10-1	trp5-48		his1-7	lys1-1	ade2-1	hom3-10
XV365-3A	α	rad10-1	trp5-48	arg4-17	his1-7		ade2-1	hom3-10
XV365-4A	α	rad10-1		arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV366-2A	α		trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV366-3A	a	rad10-1	trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV366-4A	α		trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV366-5A	α		trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV366-6A	a	rad10-1	trp5-48	arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV362-3A	a			arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV362-5A	α	rad18-1		arg4-17	his1-7	lys1-1	ade2-1	hom3-10
XV362-6A	a	rad18-1		arg4-17	his1-7	lys1-1	ade2-1	hom3-10

represent the inability to ferment maltose and galactose. In all cases, the locus is defined by three letters followed by a number, and the particular allele referred to, when assigned, is indicated by the second number.

Media

YEPD, complete medium, consists of 1% Bacto-Yeast Extract, 2% Bacto-Peptone, 2% dextrose, and 2% Bacto-Agar. It is used for maintenance of stock cultures, viability assays in mutation rate experiments, and for survival plating after exposure to ultraviolet radiation.

YEPG, used to eliminate "petite" colonies, consists of 1% Bacto-Yeast Extract, 2% Bacto-Peptone, 2% glycerol, and 2% Bacto-Agar.

Minimal Medium plus vitamins (MV) is composed of 2% dextrose, 2% Bacto-Agar, and 0.67% Bacto-Yeast Nitrogen Base without Amino Acids.

Mortimer Complete Medium (MC), (von Borstel et al., 1971), is minimal medium plus vitamins, supplemented with the following: arginine, lysine, adenine, methionine, tryptophan, uracil, and histidine, each at a concentration of 20 mg/liter; serine at 375 mg/liter; leucine at 30 mg/liter; and threonine at 350 mg/liter. MC is used as the plating medium in "lassie" tests (to be described below).

Omission media consist of MC minus one of the supplements. The kinds of omission media used were: -TRP, -ARG, -LYS, -HIS, -ADE, and -THR, which represent MC lacking tryptophan, MC lacking arginine, MC lacking lysine, MC lacking histidine, MC lacking adenine, and MC lacking threonine, respectively. Omission media are used in screening for nutritional genetic markers by the method of replica plating and

in complementation tests.

Sporulation Medium, FSM, is used for sporulating diploid strains. It contains 0.98% potassium acetate, 0.1% dextrose, 0.25% Bacto-Yeast Extract, and 1.5% Bacto-Agar, supplemented with all the amino acids as for MC.

Growth limiting liquid media (von Borstel et al., 1971), used for the mutation rate experiments, consists of MC minus agar with either lysine or histidine (depending upon which reversion rate is being measured) present in limiting concentration: 1.0 μg/ml for lysine; 0.2 μg/ml for histidine. The limiting amino acid is inoculated into the autoclaved liquid medium immediately preceding an experiment. Limiting lysine medium also contains an adjusted concentration of adenine (5.0 μg/ml). Limiting histidine medium contains 60 μg/ml of lysine.

The following media were used only for the cross of XV362-5A with XV185-14C:

Presporulation Medium II (Roth and Halvorson, 1969) contains 0.67% Bacto-Yeast Nitrogen Base without Amino Acids, 0.1% Bacto-Yeast Extract, and 1% potassium acetate in a liter of pthalate buffer (pH=5.0).

The Sporulation Medium consists of 1% potassium acetate and 7.8% mannitol, plus adenine, arginine, histidine, lysine, threonine, methionine, and tryptophan at the same concentrations as for MC.

METHODS

Replica Plating

The technique of transferring cells from a "master" YEPD plate by means of sterile velvet onto the various types of omission media was

used in screening for nutritional genetic markers.

Ultraviolet Light Survival Curve Experiments

Ultraviolet radiation was administered at an incident energy of approximately 300 ergs/mm^2/min. The source was a low pressure mercury vapor lamp, General Electric No. G30T8. The lamp was at a distance of 52 cm and was shielded by a wooden board, except for an aperture, 10 cm in diameter, below which the irradiations were carried out.

Cells from a single colony isolate of a particular strain were suspended in 5 mls of standard lab buffer (1/15M solution of monobasic potassium phosphate, pH=4.7) and adjusted to 5×10^6 cells/ml. Dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} were made, and 0.5 ml of the appropriate dilution was plated onto YEPD. The plates of media were irradiated, four at a time with lids removed, within approximately one hour of plating. Plates were irradiated for 0, 1, 3, or 5 minutes. All irradiation was performed in a darkened room and plates were incubated in the dark at 26°C. After five days, colony counts were made and the survival fraction at each dose for each strain calculated as $\frac{\text{the number of colonies obtained at a particular dose}}{\text{the number of colonies on the unirradiated control plate}}$. For each experiment carried out, three control strains were also irradiated: X1687-12B, a UV-insensitive strain whose survival curve represents the wild type; KC372, a highly UV-sensitive strain bearing the rad18-2 radiosensitivity allele; and KC376 rad52-1, an X-ray sensitive strain which is only weakly sensitive to UV. The dilutions used in these experiments were as follows:

	Time of Irradiation (min)			
Strain	0	1	3	5
X1687-12B	10^{-4}	10^{-4}	10^{-3}	10^{-2}
KC372	10^{-4}	10^{-1}	10^{-0}	-
KC376	10^{-4}	10^{-3}	10^{-1}	10^{-0}
tested strains	10^{-4}	10^{-1}	10^{-0}	10^{-0}

UV "Spot" Tests

For quick screening of strains for sensitivity to ultraviolet light, a qualitative UV test was used. Suspensions of cells to be tested were made up and adjusted to 5×10^6 cells/ml. Small drops of these suspensions, dispensed by means of sterile pasteur pipets, were inoculated onto solid YEPD media. Control strains were treated similarly.[1] These plates were then exposed to ultraviolet light for 0, 1, 3 or 5 minutes following the same procedure as described above for survival curve experiments. After two days' incubation, it is possible to determine whether or not a strain is UV-sensitive by scoring the presence or absence of growth at the different doses. If a strain is insensitive to UV, it will grow well on both unirradiated and irradiated plates. If it is sensitive to UV, there will be no growth, except possibly for a few surviving colonies, on the irradiated plates. Most UV-sensitive strains are nearly "wiped out" after a one minute dose and are clearly negative as regards growth after three minutes. For less UV-sensitive strains, it may take a dose of

five minutes to inhibit growth.

For some of the strains, actually UV survival curves were also done.

Matings

Cells of strains of opposite mating type were grown overnight on fresh YEPD and crossed early the next morning by manual mixing of cells with a sterile wooden applicator. Mating was allowed to proceed for approximately four to six hours, after which time cells were examined under a microscope for the presence of dumbbell-shaped zygotes. Suspensions of the mating mixtures were made in 5 mls buffer, adjusted to 5×10^6 cells/ml concentration, and plated on YEPD at dilutions to give single colonies. The plates were incubated for three days at 26°C.

Sporulation

Suspected diploid colonies were picked and restreaked on a new YEPD plate. After one day's growth, the colonies were replicated onto FSM and incubated for two or three days. Cells from FSM were then examined microscopically. If spore-containing asci were present in fairly large proportions, cells were scooped up from FSM by means of a sterile wooden splint and placed in 0.03 ml of a 1 in 20 dilution of glusulase enzyme to water. After ten minutes, the digestion of the ascus wall was stopped by the addition of 3 mls sterile water. This suspension was then sonicated, one minute at a time followed by cooling, for a total of three minutes at 50 watts, using an Ultrasonic Devices (Heat Systems Company, Melville, N.Y.) sonicator, model 516-692-9590. The purpose of the sonication treatment was to separate

the spores from each other. Following sonication, suspensions were once again microscopically examined to ensure the presence of free spores. Cell counts were then made and appropriate dilutions of the sonicated mixture plated out on YEPD and incubated for three to four days at 26°C.

Spores from the cross of XS774-5D rad1-1 with XV185-6A were obtained from tetrads through ascus dissection, by the technique of Johnston and Mortimer (1959).

Spheroplast Formation

To sporulate XV424, a slightly modified technique from the one described above was used. Two single colony diploid isolates with the highest sporulation frequency on FSM (approximately 26% and 20%) were selected. Cells from these isolates, growing on YEPD, were suspended in 25 mls Presporulation Medium II and shaken at 250 RPM on a New Brunswick Scientific Company Rotary incubator-shaker, model R27, at 24°C for approximately 24 hours. After this time, cells were counted and found to be of the order of 1×10^7 cells/ml, that is, at the end of log phase. The suspensions were then centrifuged for five minutes at 2727 RPM in an International Clinical Centrifuge. The supernatant Presporulation Medium was poured off, and the pellets washed in 5 mls distilled water, vortexed, recentrifuged for five minutes and resuspended in 5 mls sodium thioglycolate (0.5M) containing 0.1M Tris buffer (pH=8.8). These suspensions were then shaken for 35 minutes, centrifuged, and washed twice. Two mls 0.7M mannitol and 0.5 ml glusulase were then added and the suspensions shaken for

17 hours. The spheroplasts were collected by centrifugation, washed in 5 mls cold mannitol (0.7M), and recentrifuged. The pellets were each suspended in the sporulation medium containing mannitol and allowed to shake for four days in a 125 ml flask. The sporulation frequency was then checked, the sporulation medium removed by centrifugation for 10 minutes, and the pellets washed in 10 mls water to lyse the vegetative diploid cells. Each pellet was then resuspended in 3 mls of water and sonicated.

Complementation Tests

The six tester strains, whose genotypes are given in Table 2, were: X464-20C, X464-1A, XV185-6A, XV185-14C, XV185-4A, and XV185-6D.

Tester strains and the strains of unknown mating type were streaked out from one end of the plate to the other, six to a plate, on separate YEPD plates, grown overnight, and then replicated at right angles to each other onto -LYS, -ADE, and -HIS media. Diploids form at the intersections where cells of opposite mating type meet.

A strain, for example, of mating type α and having the markers ade2-1, his1-7, and lys1-1, will not grow on -ADE, -HIS, and -LYS media unless it mates with a tester strain of opposite mating type and wild type at the particular locus mutant in the unknown spore. Such a strain would only mate with a strains, and on -ADE would show growth (or complementation) only with X464-1A; on -HIS would complement with X464-1A and XV185-6D; on -LYS would complement with X464-1A. Thus, it is possible to determine the mating type and the mutant alleles carried by an unknown strain culture.

Measurement of Reversion Frequency

Spontaneous reversion from auxotrophy to prototrophy is studied by means of the 1000-compartment fluctuation test (von Borstel et al., 1971).

Suspensions used in these "box" experiments were generally the same as the ones which had been UV tested. Suspensions of 5×10^6 cells/ml were made up from cells grown three days on YEPD and 0.5 ml is inoculated into 1330 mls of limiting liquid medium. The medium, stirred continuously with a magnetic stirrer, is delivered in 1 ml aliquots into ten 100-compartmented culture boxes by means of a Brewer Automatic Pipetting Machine, model No. 60453 (Baltimore Biological Laboratory). The calibration of the syringe is checked before and after each experiment by delivering 20 squirts into a 25 ml graduated cylinder. Also, 1 ml of the medium is squirted into 4 mls buffer and 0.5 ml of this suspension plated on each of two YEPD plates to determine cell viability. After being filled, each box is sealed with masking tape and incubated at 26°C.

Revertant colonies begin to appear at about the third day, after growth of the inoculated cells has reached a plateau. Revertants to lysine (or histidine) independence grow and multiply even after the supply of lysine (or histidine) is depleted. The date of appearance of the first revertant colony in each compartment is recorded on the box lid daily, starting from the third day. Lysine reversion experiments, were scored for 14 days; histidine experiments for 12 days.

The frequency of revertants already present in the cell suspension is determined by plating on two plates of -LYS (or -HIS) media,

a few days before the start of an experiment. At the end of an experiment, the number of cell divisions which occurred before the limiting requirement was depleted is determined by counting microscopically the number of cells in two compartments without reversions, chosen in each box according to a prearranged pattern. The total number of revertant-containing compartments for each day is recorded per box.

Revertant Analysis

The revertants (one per compartment) were also picked for further analysis: lysine revertants onto -LYS; histidine revertants onto -HIS. For most experiments, and definitely for controls, all lysine revertants were picked. For all histidine experiments, two plates (approximately 72 revertants) were picked for analysis. The revertants were picked from the box compartment and transferred onto -LYS or -HIS plates by means of sterile pasteur pipets. The plates were incubated for five days, to allow for cell growth, and then replica plated onto YEPD, MV, MC and the various omission media.

Computation of Mutation Rates

The number of revertant colonies in independent compartments is assumed to follow a Poisson distribution. To calculate the reversion rates by this method, the number of compartments containing no revertant colonies (the P_0 term) is used. The method of computation is as follows (von Borstel et al., 1971):

Let N be the number of compartments in an experiment, and N_0 the number of compartments without revertants. From

the zeroth term of a Poisson distribution we have

$$e^{-m} = N_0/N$$

where m equals the average number of mutational events (not mutants) per compartment. Most of these mutational events are due to new mutations arising during the growth of the cells in the limiting medium, but some are due to mutants, present in the inoculum. We correct for this "background" by

$$m_g = m - m_b$$

where m_b is the average number of mutants per compartment in the inoculum (as determined by direct plating), and m_g is the corrected average number, i.e., the mutational events occurring during the growth in the compartments. This can be converted to the mutational events per cell per generation, M, by

$$M = m_g/2C$$

where C is the number of cells per compartment after growth has ceased in the limiting medium. The factor of two in the denominator is necessary because the number of cell generations in the history of a culture is approximately twice the final number of cells. Actually, the proper value for this numerical factor depends upon the point(s) in the cell cycle at which growth is terminated in the limiting medium and also upon the distribution of mutation production over the cell cycle. Since it enters only as a scale factor in all mutation rate calculations, relative mutation rates are unaffected by the value used.

This method for determining mutation rates is due to Luria and Delbruck (1943). The principal advantage of the method is that the results are not affected by many types of selection. Since we only score the presence or absence of a mutational event in a culture, it is clearly irrelevant whether the mutants grow faster or slower than non-mutants.

In those experiments where the mutants are further analyzed into categories the mutation rate may be partitioned by

$$M_i = f_i M,$$

where M_i is the mutation rate (per cell per generation) for the ith category and f_i is the fraction of the mutants tested which were found to be in the ith category.

A computer program was used to obtain values for the mutation rates.

The "Lassie" Test

"Lassie" tests were carried out on several UV-sensitive and UV-insensitive strains from each cross. Suspensions of selected strains are plated at a concentration of 5×10^6 cells/ml, 0.5 ml per plate, on two plates of each of -LYS and MC media. The purpose of the -LYS media is to determine whether any background revertants to lysine independence are already present in the suspension. Plating on MC provides a qualitative estimate of the mutator activity of a strain. Within one to two days of plating, a thin confluent background of mutant cells form on the surface of the MC media. However, exhaustion of the lysine in MC eventually will limit the growth of the mutant cells plated, whereas revertants to lysine independence continue to grow. Within three to four days, therefore, single revertant colonies begin to appear, visible against the background growth. The plates are incubated for 10 days at 26°C, after which time colony counts are made. The number of colonies on an MC plate indicates whether or not a strain is a mutator.

RESULTS

Genotypes of Strains Bearing the *rad* Alleles

Nutritional Markers. Replica plating single colony isolates of the radiation-sensitive strains (Table 1) confirmed their expected genotypes: all were wild type with respect to nutritional genetic markers with the exception of the *rad3-12*, *rad8-1*, *rad18-2*, and *rad52-1* allele-bearing strains. The strain bearing the *rad3-12* allele was found to be auxotrophic for histidine in addition to *ade2-1*. Complementation tests revealed that the histidine marker was neither *his1* nor *his5*. Consequently the marker has been designated simply as *his* in Table 1.

Sensitivity to Ultraviolet Light. A UV-survival curve was drawn for each of the UV-sensitive strains. In the Appendix, Table A1, are given the dilutions used and the colony counts and surviving fractions obtained at different doses for the strains bearing the *rad* alleles involved in the excision repair pathway (*rad1-1*, *rad2-2*, *rad3-12*, *rad4-3*) and a related pathway (*rad5-1*). Figure 1 shows the survival curves obtained. It can be seen that *rad1-1*, *rad2-2*, and *rad4-3* show essentially the same high sensitivity to UV, whereas *rad3-12* and *rad5-1* are much less sensitive. Table A2, Appendix, gives similar information about the UV survival of *rad* alleles involved in other, less well-known repair pathways. From Figure 2, it is obvious that the *rad18-1* and *rad18-2* alleles are quite sensitive to the killing effect of UV light, whereas *rad10-1* is slightly less sensitive

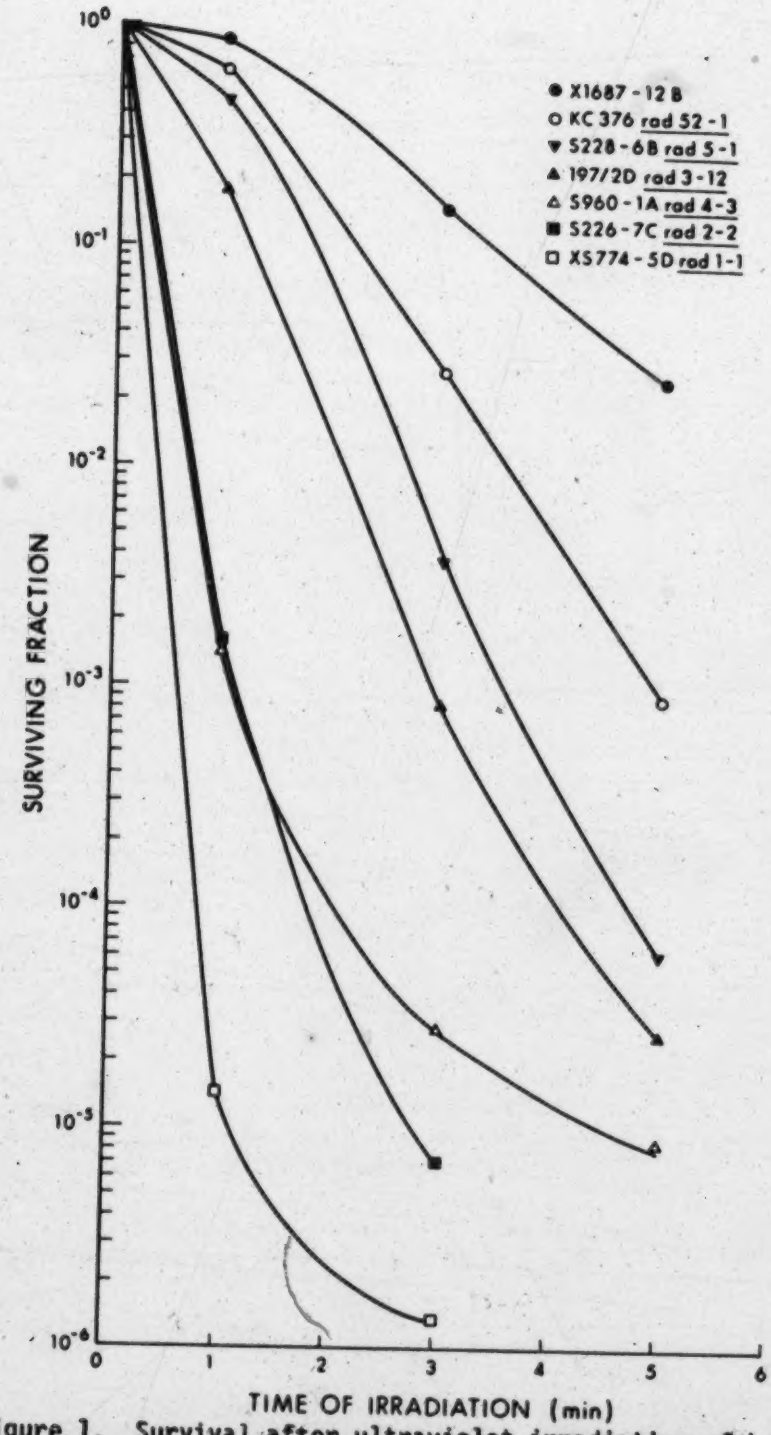

X1687 - 12 B
KC 376 rad 52 - 1
S228 - 6B rad 5 - 1
197/2D rad 3 - 12
S960 - 1A rad 4 - 3
S226 - 7C rad 2 - 2
XS774 - 5D rad 1 - 1
SURVIVING FRACTION
TIME OF IRRADIATION (min)

Figure 1. Survival after ultraviolet irradiation of haploid strains bearing the rad alleles involved in the excision repair and related pathways.

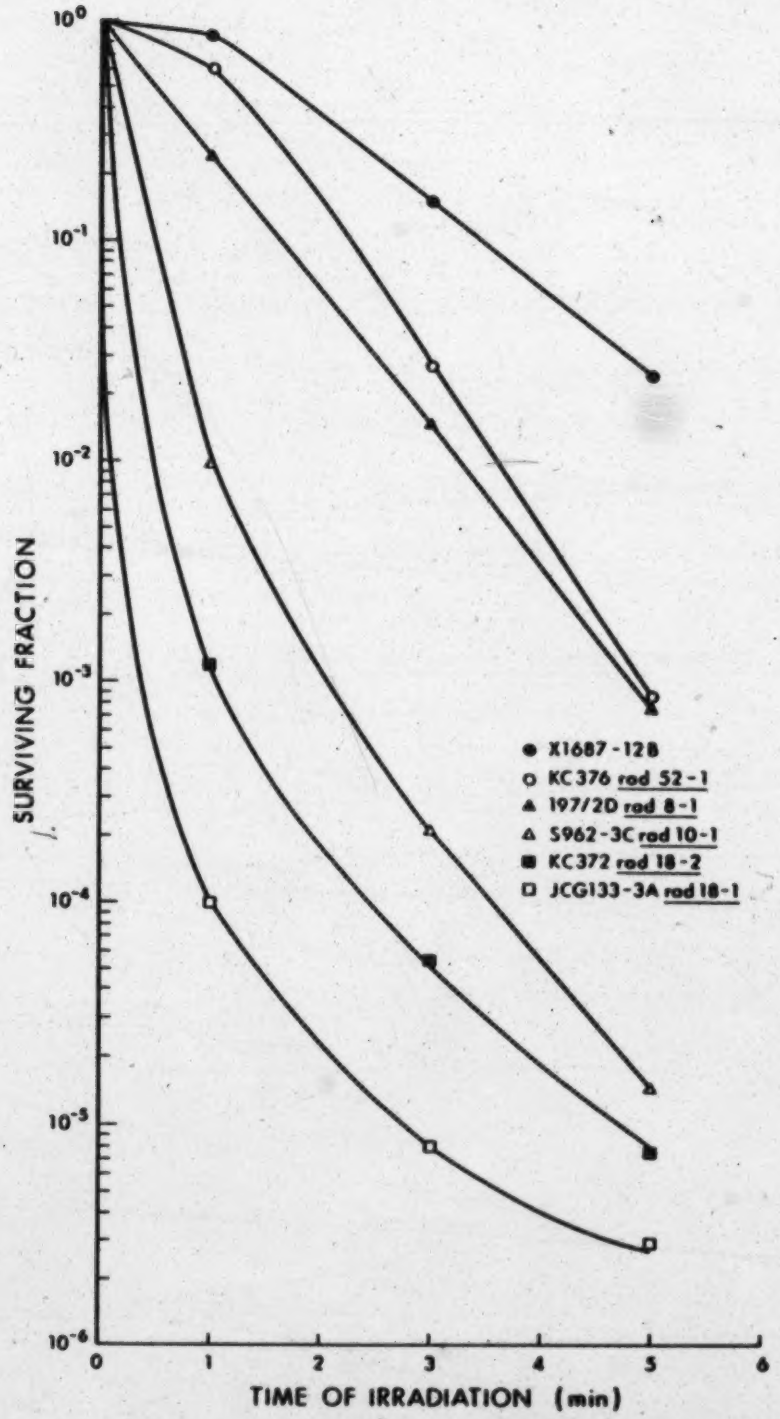


Figure 2. Survival after ultraviolet irradiation of haploid strains bearing the rad alleles involved in other unknown repair pathways.

and rad8-1 is only weakly UV-sensitive. The survival curves for the strains bearing the rad3-12, rad5-1, and rad8-1 alleles were obtained by plating at the dilutions used for the strain KC376 rad52-1 which is only slightly UV-sensitive. Plating them at the most dilute concentrations, generally used for UV-sensitive strains, gave confluent cell layers which were impossible to count. Thus, it seems that rad3-12, rad5-1, and rad8-1, in that order of decreasing sensitivity, are only slightly more sensitive to UV than KC376. It will also be noted that the colony counts, upon which these UV survival curves are based (Appendix, Tables A1 and A2), were often quite low, especially on the five minute dose plates, thus making the curves subject to some error. However, all the curves shown here correspond favorably to those obtained by the original investigators (Nakai and Matsumoto, 1967; Snow, 1967; Cox and Parry, 1968; Resnick, 1969; Parry et al., 1972).

Introduction of Genetic Markers into the UV-Sensitive Strains

Random Spore Analysis. In order to measure the spontaneous reversion rates from auxotrophy to prototrophy, nutritional genetic markers had to be introduced into most of the UV-sensitive strains. This was achieved by outcrossing the single colony isolate, on which the UV survival curve had been done, with a standard laboratory control strain of opposite mating type. In the earlier crosses performed, XV169-12A or XV169-15A were used. However, as cells from these strains tend to clump together making it difficult to obtain an accurate cell count, the strains XV185-6A or XV185-14C, in which

this undesirable trait has been eliminated, were used in later crosses. The standard strains all carry the markers trp5-48, ade2-1, arg4-17, lys1-1, his1-7, and hom3-10.

Table 3 lists all the crosses which were made. It will be observed that the strains bearing the rad1-1 and rad2-2 alleles were each mated on two separate occasions. The reason for this was that too few UV-sensitive and/or UV-insensitive strains were obtained from the first cross in each case, and, therefore, another single colony isolate of the original rad-bearing strain had to be outcrossed. It will also be noted that one of the spores derived from the diploid XV365 and carrying the rad10-1 allele, namely XV365-3A, was also outcrossed again and second generation spores obtained.

After sporulation of the diploids, red spore colonies were selected. These were already known to be mutant for adenine, as a mutation in the ade2 gene causes the formation of a red pigment. These colonies were then restreaked onto YEPD, grown for one day and replica plated onto YEPD, MV, -TRP, -ARG, -HIS, -LYS, -ADE, -THR, YEPG, and MC media. Only those colonies which carried the appropriate markers (histidine and lysine being mandatory) were selected and tested for sensitivity to UV light by means of the "spot" test. This selection method thus yielded strains from each diploid which had all or most of the six nutritional requirements and were either UV-sensitive or UV-insensitive.

UV-Sensitivity. UV "spot" tests give a fairly accurate representation of UV-sensitivity. Strains bearing the alleles rad1-1, rad2-2, rad4-3, rad10-1, rad18-1, and rad18-2 all showed sensitivity

after a one minute UV dose, in agreement with their high UV-sensitivity as shown by their survival curves. A three minute radiation dose was required for strains bearing rad3-12 and rad5-1 to show UV-sensitivity, and three to five minutes for rad8-1 strains. This is in accord with the fact that these three rad alleles confer less UV-sensitivity.

For some of the strains, actual UV survival curves were also done and these are shown in Figures 3 to 7. The surviving fractions and actual colony counts are given in the Appendix, Tables A3 to A7. A comparison of the curves obtained with these auxotrophic UV-sensitive strains against those of their radiation-sensitive parents shows almost complete superimposibility, with slight errors attributable to low colony counts on three and five minute-irradiated plates. The variation of XV365-4A rad10-1 is probably due to the effects of genetic background.

Complementation Tests. Complementation tests were also done in order to determine the mating type of each strain and to ensure the strains selected were actually haploid and not diploids having arisen from the mating of two haploid spores not properly separated by means of sonication. Where a UV-insensitive strain carried the same markers as its UV-insensitive parent, it was eliminated and only strains of opposite mating type from the parent were chosen for use as controls. The complementation patterns revealed that all strains selected carried the lys1-1, ade2, and his1-7 markers since no complementation occurred with tester strains carrying these markers. Any strains which would not mate were eliminated.

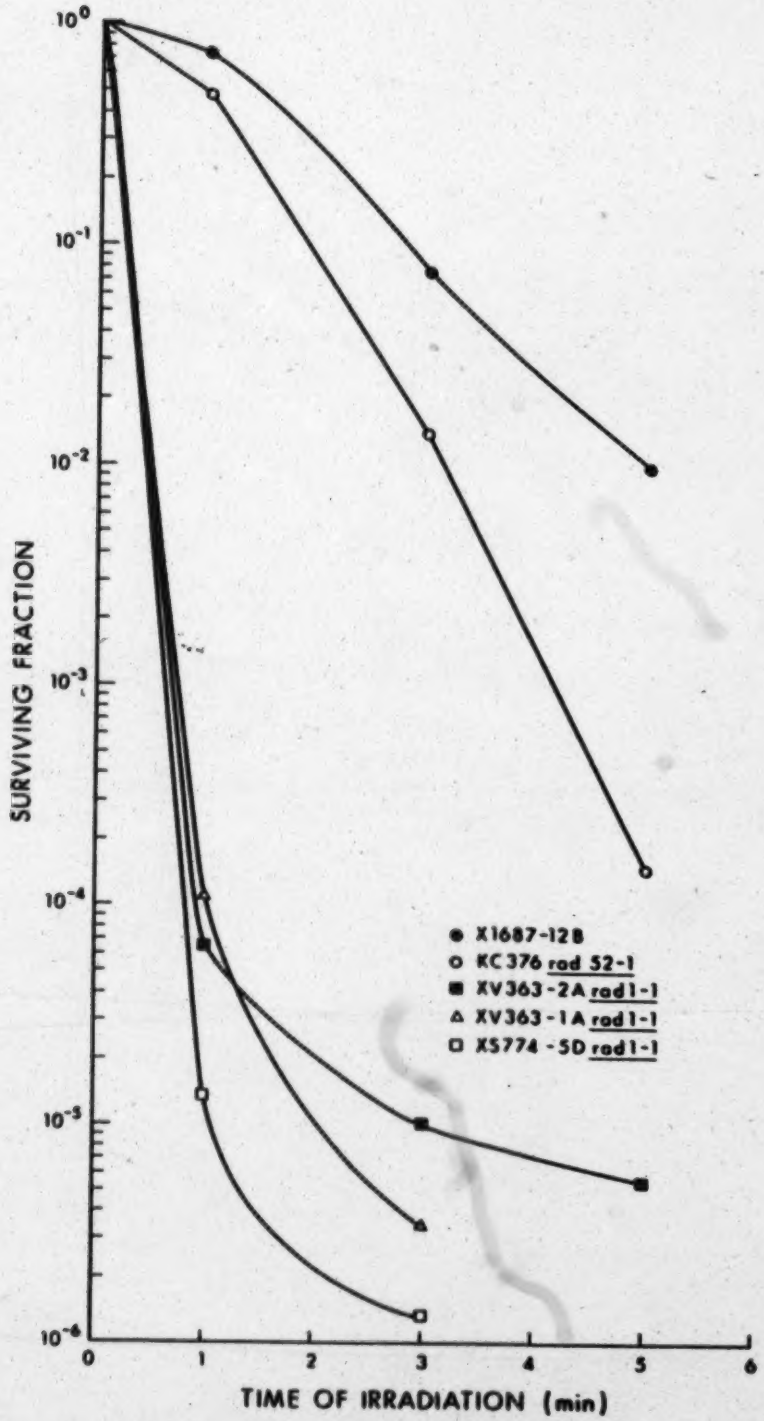


Figure 3. Survival after ultraviolet irradiation of haploid strains bearing the rad1-1 allele.

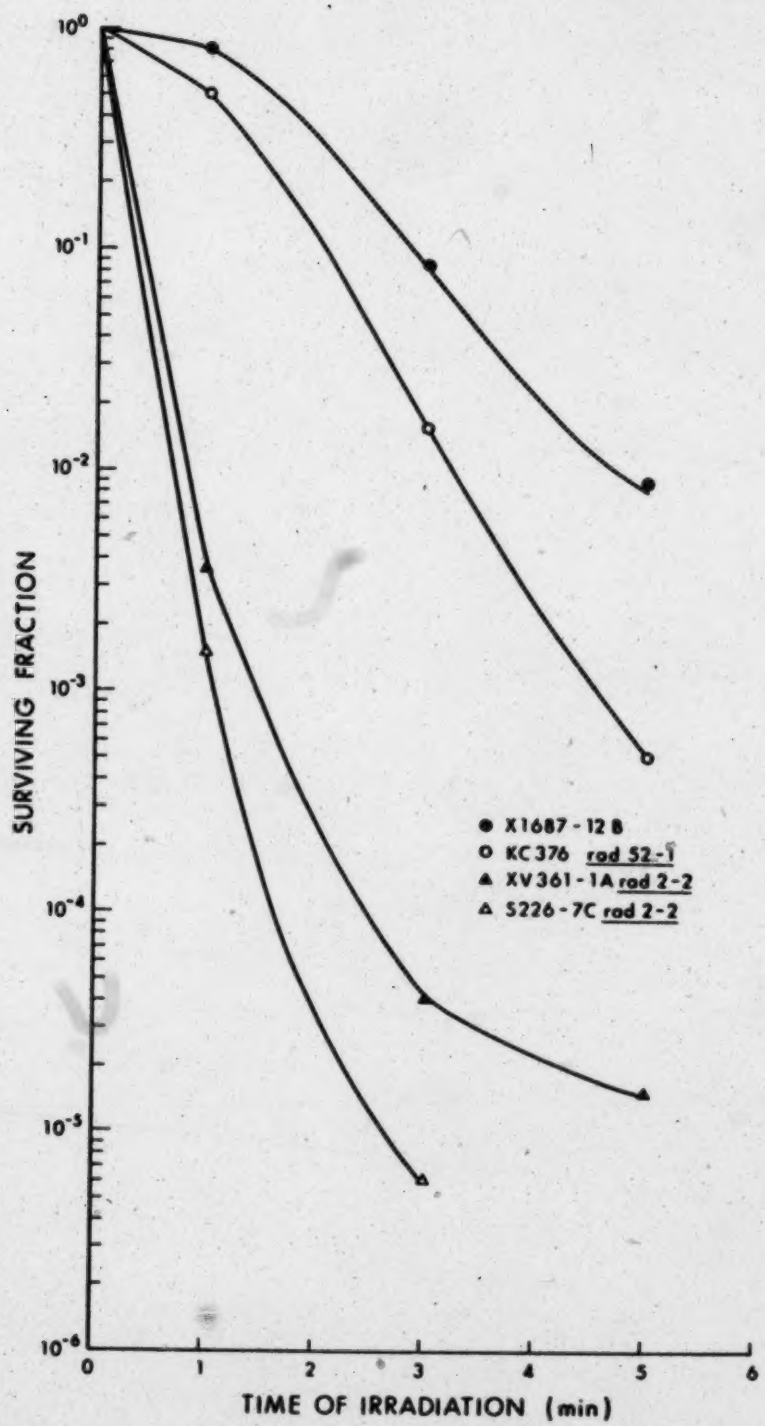


Figure 4. Survival after ultraviolet irradiation of haploid strains bearing the rad2-2 allele.

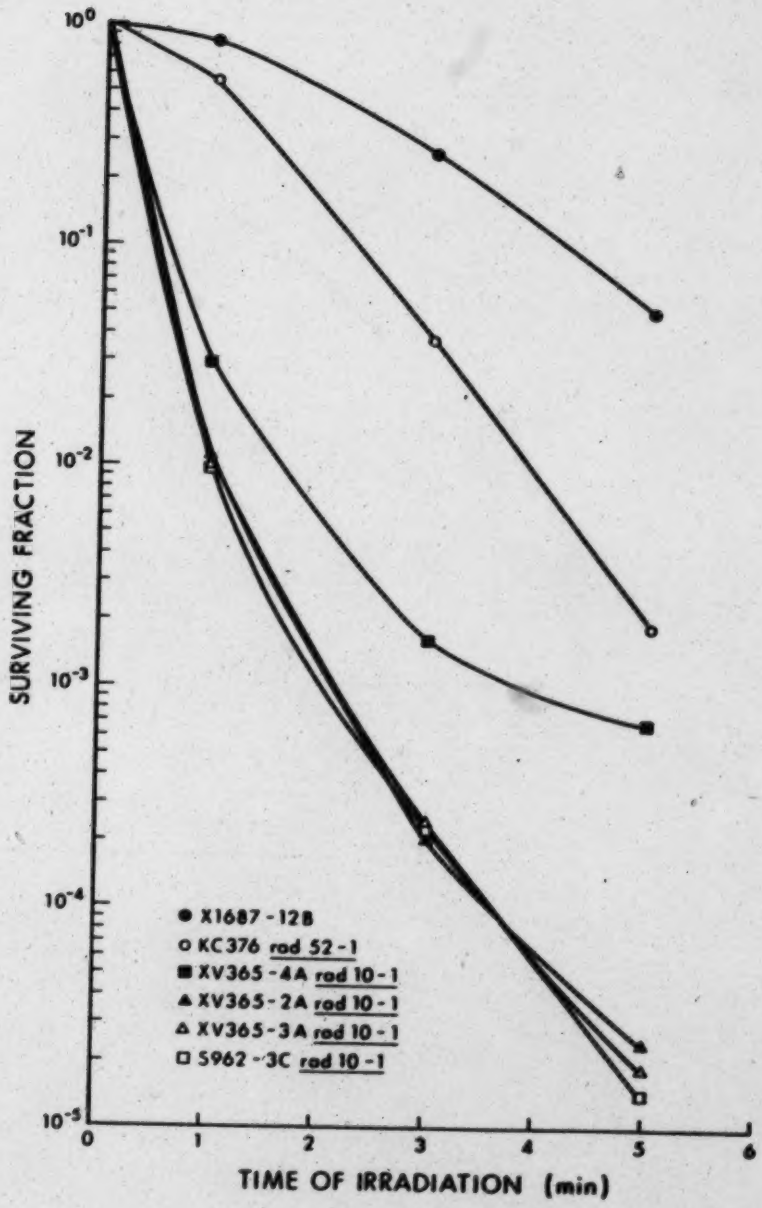


Figure 5. Survival after ultraviolet irradiation of haploid strains bearing the radlO-1 allele and derived from the diploid XV365.

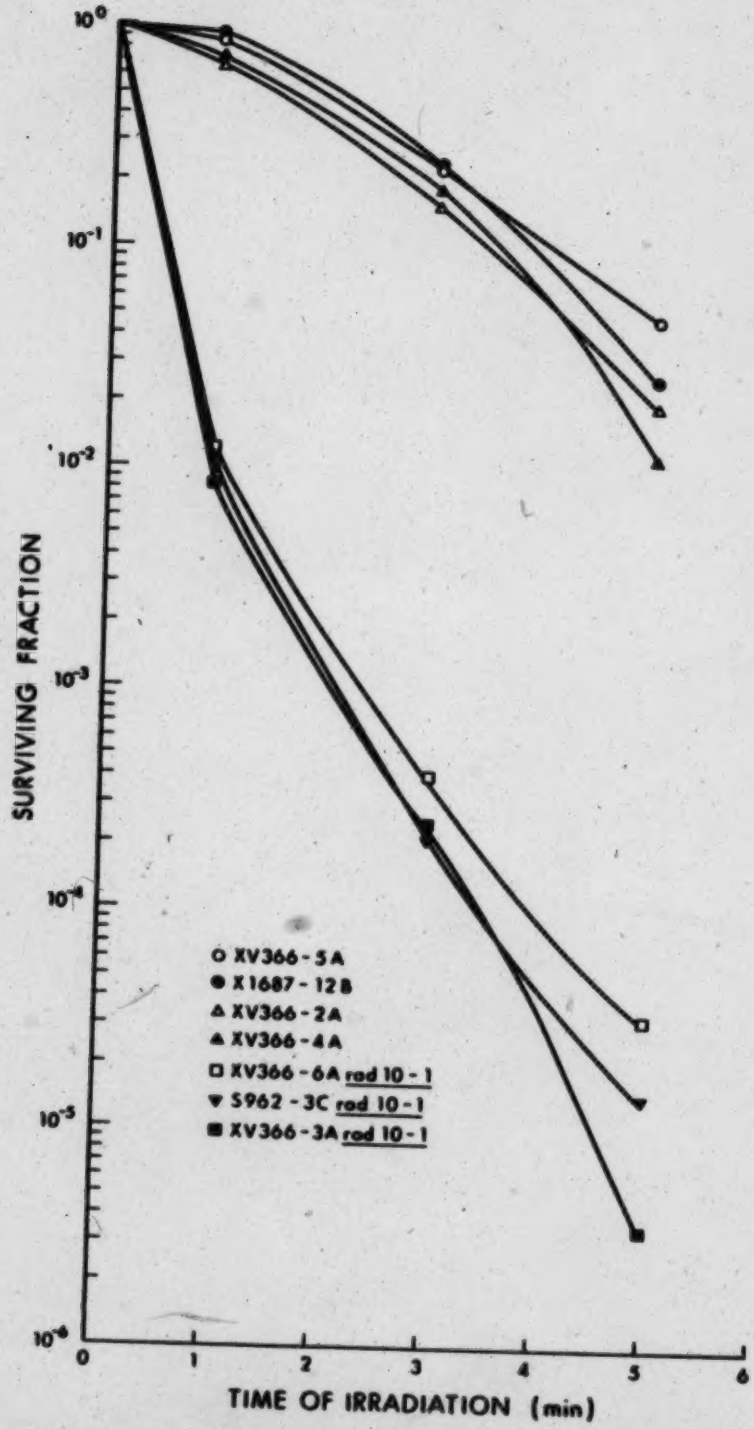


Figure 6. Survival after ultraviolet irradiation of haploid strains bearing the rad10-1 or its wild type allele and derived from the diploid XV366.

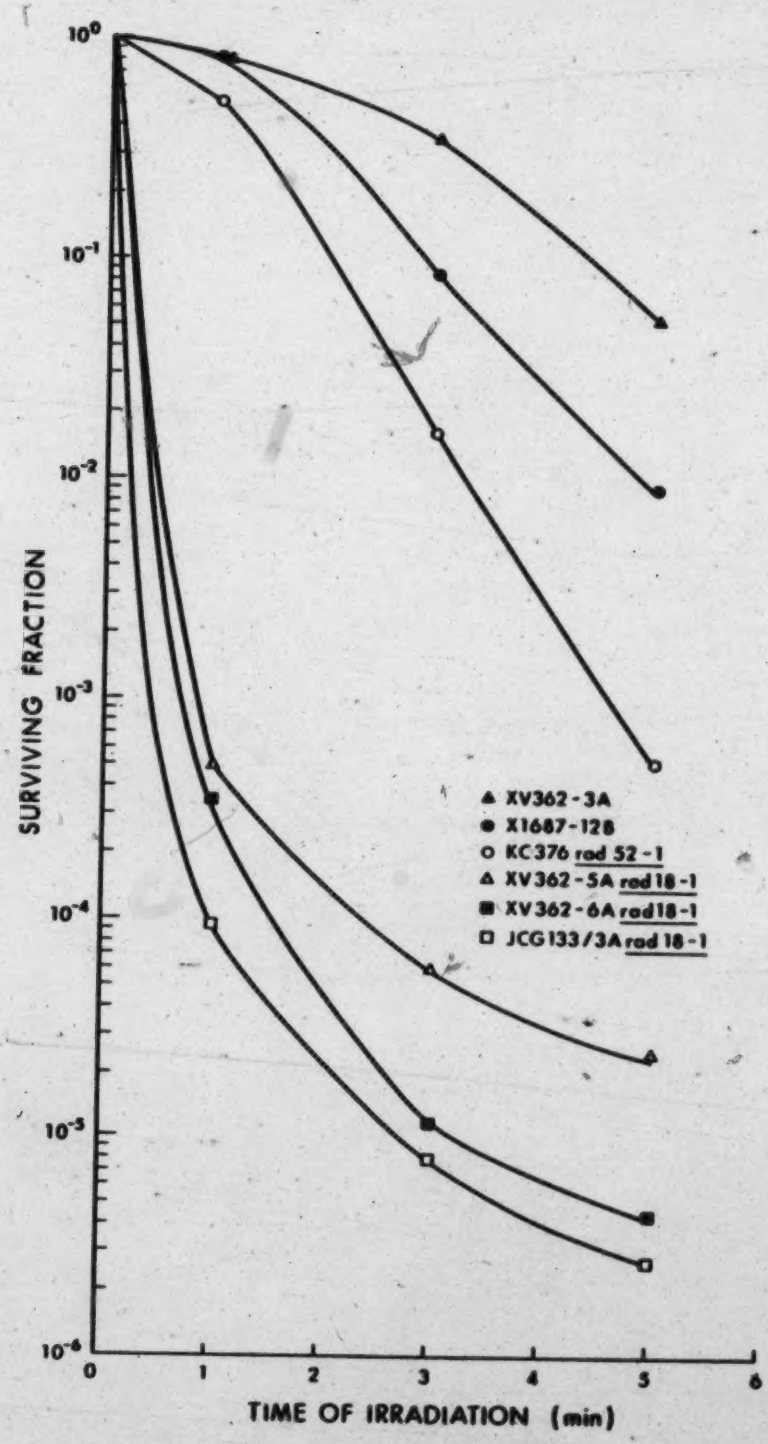


Figure 7. Survival after ultraviolet irradiation of haploid strains bearing the rad18-1 or its wild type allele.

Determination of Spontaneous Mutation Rates

Kinetics. The spontaneous reversion rates to lysine and histidine independence in several of the auxotrophic rad allele-bearing daughter strains were then measured. A mutant lysine (or histidine)-requiring strain is inoculated into liquid medium containing a limiting amount of lysine (or histidine). For each strain tested, small 1 ml cultures are started in each of 1000 box compartments. The mutant cells in a compartment grow and divide only until the lysine (or histidine) supply is exhausted, whereas revertants to lysine (or histidine) independence which occur during the growth of the mutant culture continue to grow. Because the growth of mutant cells to saturation is not permitted to take place, the revertants can readily be seen forming visible colonies in the compartments.

The first revertant colonies appear after three to four days of incubation; that is, after the time required for a single cell to make a colony under anaerobic conditions at room temperature (von Borstel et al., 1971). Althought a slight amount of variation can exist between different strains, most of the lysine revertants have appeared by six to eight days. After this time slight increases observed in the number of revertants are probably due to a small amount of cell division resulting from cell death and reutilization of nutrients (von Borstel et al., 1971). In contrast, however, the number of histidine revertants often keeps increasing even up to the twelfth day, as will be discussed below.

Types of Revertants. Reversion to lysine independence can occur either by a reversion at the lys1 locus itself or by a forward mutation

at a supersuppressor locus (von Borstel et al., 1971). The first situation results only in lysine independence. A mutation at a supersuppressor locus, however, will also result in suppression of the other nonsense mutant alleles carried by the strain: trp5-48, arg4-17, and ade2-1. Locus reversions can easily be distinguished from suppressor mutations by the color of the revertant (Schuller and von Borstel, 1972). Locus revertants, since they remain auxotrophic for adenine and ade2 mutants accumulate red pigment, will turn red once the adjusted adenine concentration in the medium is depleted. Supersuppressor mutations, since they suppress ade2-1, will be white. Most supersuppressors are of this Class I type (Gilmore, 1967; Hawthorne and Mortimer, 1968), but a few may be Class II Set 1 (suppressor 11). The latter variety do not suppress ade2-1. Picking the revertants onto -LYS and then replica plating them onto the various omission media also distinguishes locus and supersuppressor mutations and permits classification of supersuppressors. Locus revertants often represent less than 10% of the total number of revertants in control (non-mutator) strains, and, as they are based on low numbers, locus reversion rates are subject to some degree of error. Most reversions are of the supersuppressor type.

Reversions to histidine independence, on the other hand, are believed to occur through internal missense suppression (Korch and Snow, 1973). All histidine revertants are white phenotypically as only his1-7 is suppressed, and that by another base substitution in the his1 gene itself.

Effects of Radiosensitivity Alleles Involved in the Excision Repair Pathway on Reversion Rates to Lysine Independence. The rates of reversion to lysine and histidine independence were determined for several haploid strains, each carrying a mutant allele of one of the four rad loci involved in excision repair. The genotypes of these strains are given in Table 4. The reversion rates to lysine independence, calculated from the number of compartments without revertants present on day 10 of incubation (von Borstel et al., 1971), are shown in Table 6. The total number of compartments or 1 ml cultures upon which these rates are based and the final cell liter are also given. The number of compartments with one or more revertants (given in the table as the number of revertants) as well as the locus and supersuppressor (SS) reversion rates are shown and the two latter are summed to give the overall total mutation rate.

The reversion rates of UV-insensitive strains, derived from each cross of a radiation-sensitive strain, were also measured and served as controls. Before examining the mutation rates of the radiation-sensitive strains, it is worthwhile to examine the range of values observed in the UV-insensitive strains. The average total lysine mutation rate, for all UV-insensitive strains derived from crosses involving the rad1 to rad4 alleles, is 2.9×10^{-8} mutational events per cell per generation (average locus mutation rate is 0.5×10^{-8} mutational events per cell per generation; average supersuppressor mutation rate is 2.4×10^{-8} mutational events per cell per generation). The range of values of total mutation rates to lysine independence obtained for all the UV-insensitive strains tested in this study

TABLE 6. Spontaneous reversion rates to lysine independence in haploid strains bearing a rad or its wild type allele involved in the excision repair pathway

Expt. No.	Strain	rad Allele	Cells/ml (x 10^{-6})	Total Compts.	No. of Revertants Locus	No. of Revertants SS	Mutation Rate ($\times 10^{8}$) Total	Mutation Rate ($\times 10^{8}$) Locus	Mutation Rate ($\times 10^{8}$) SS
43.16	XV363-11A		1.8	997	15	88	2.9	0.4	2.5
50.27	XV421-5D		1.6	997	14	79	3.2	0.5	2.7
50.29	XV421-15B		1.6	1000	15	80	3.1	0.5	2.6
	Average:						3.1	0.5	2.6
43.12	XV363-1A	rad1-1	1.3	998	21	109	5.1	0.8	4.3
47.01	XV363-1A	rad1-1	1.5	997	21	128	4.5	0.7	3.8
43.14	XV363-2A	rad1-1	1.4	995	25	124	5.4	0.9	4.5
47.02	XV363-2A	rad1-1	1.5	1000	22	84	3.9	0.8	3.1
43.17	XV363-10A	rad1-1	1.5	999	18	80	3.4	0.6	2.8
50.15	XV421-12A	rad1-1	1.6	1000	13	115	4.3	0.4	3.9
50.17	XV421-17A	rad1-1	1.3	801	6	44	2.0	0.3	1.7
	Average:						4.0	0.6	3.4
50.02	XV361-2A		1.7	996	30	67	3.0	0.9	2.1
50.25	XV422-3A		1.8	999	10	58	1.9	0.3	1.6
50.21	XV422-4A		1.9	999	21	84	2.9	0.6	2.3
	Average:						2.6	0.6	2.0
43.29	XV361-1A	rad2-2	1.3	1000	6	50	2.2	0.2	2.0
50.19	XV422-1A	rad2-2	1.9	1001	27	82	3.0	0.7	2.3
50.23	XV422-2A	rad2-2	2.0	1000	26	72	2.6	0.7	1.9
	Average:						2.6	0.5	2.1
50.33	XV423-3A		1.8	995	28	134	4.9	0.8	4.1
50.35	XV423-4A		2.2	998	22	103	3.1	0.5	2.6
	Average:						4.1	0.7	3.4
50.31	XV423-1A	rad3-12	1.6	998	142	280	15.5	4.9	10.6
50.37	XV423-2A	rad3-12	1.2	999	47	213	12.1	2.0	10.1
	Average:						13.9	3.5	10.4
47.14	XV418-3B		1.8	999	14	56	2.0	0.4	1.6
47.13	XV418-8A		1.8	999	4	68	2.1	0.1	2.0
	Average:						2.1	0.3	1.8
47.11	XV418-1A	rad4-3	1.8	999	15	75	2.5	0.4	2.1
47.12	XV418-2A	rad4-3	2.0	998	19	58	2.0	0.5	1.5
	Average:						2.3	0.5	1.8

varies from about 1.0×10^{-8} to 4.0×10^{-8} mutational events per cell per generation, as shown in Figure 8. These values agree with those obtained in other experiments for UV-insensitive non-mutator control strains, which differ by as much as 2.0×10^{-8} mutational events per cell per generation (von Borstel et al., 1971; von Borstel et al., 1973).

Table 6 illustrates that, while both locus and supersuppressor mutation rates in the three UV-insensitive strains (derived from two separate crosses of the original strain bearing the radl-1 allele) do not vary greatly, a certain amount of variation is observed among the UV-sensitive strains. However, some variability is also seen to exist within the same strain, as demonstrated by measurements of the reversion rates of two different single colony isolates of each of the strains XV363-1A and XV363-2A. Consequently, some variation in mutation rate within each cross and between the two crosses is to be expected. Since all the reversion rate values (both locus and supersuppressor) fall within the range of values observed for UV-insensitive strains obtained from other crosses in this study or used in other experiments (von Borstel et al., 1971), it seems as if the radl-1 allele does not significantly increase the reversion rate to lysine independence.

It can readily be seen from Table 6 that the rad2-2 allele has no effect on spontaneous reversion rate to lysine independence. The extent of variation observed in each cross between UV-sensitive and UV-insensitive strains is within the range observed for RAD2-2 strains, and there is no great difference between the two crosses. The rather

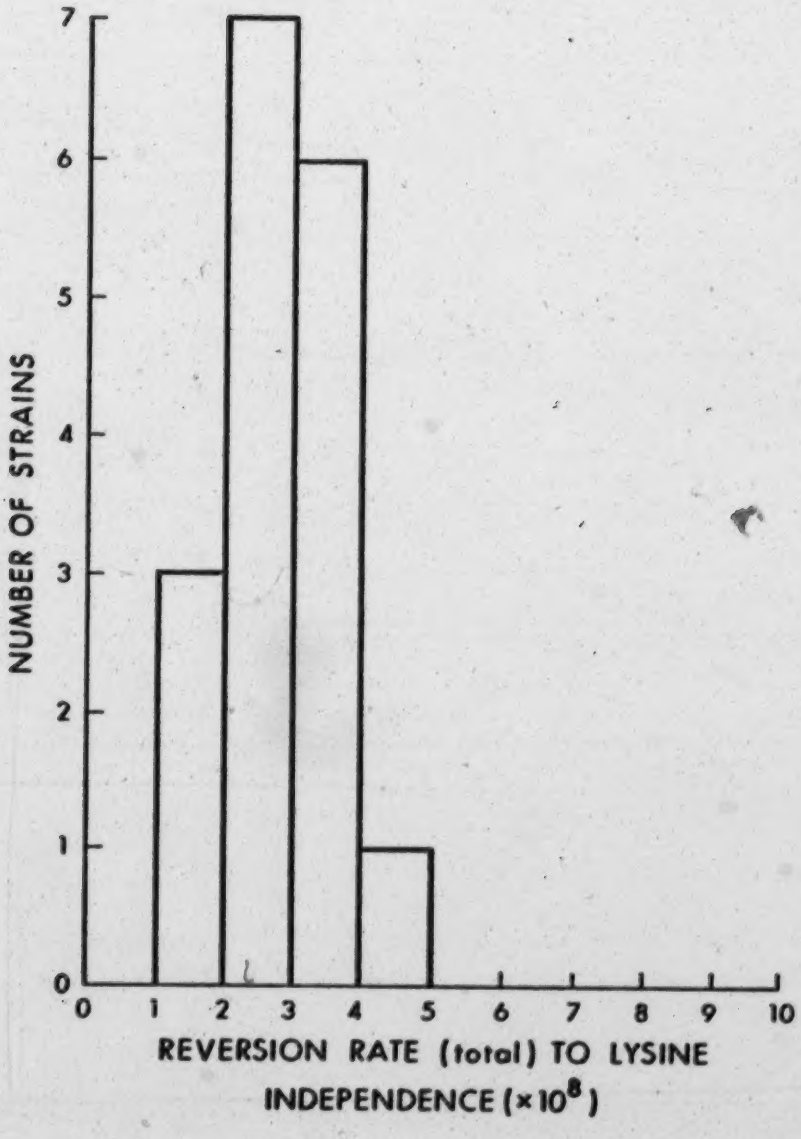


Figure 8. Frequency distribution of the (total) spontaneous reversion rates to lysine independence in strains insensitive to ultraviolet irradiation.

high locus reversion rate for the UV-insensitive strain XV361-2A may be due to scoring some supersuppressor mutations as locus reversions (see explanation under rad3-12 below). The radiosensitivity allele rad4-3 also does not increase the spontaneous reversion rate to lysine independence, as UV-sensitive strains do not differ much from the UV-insensitive strains.

The rad3-12 allele, on the other hand, increases both the locus and supersuppressor mutation rates to lysine independence by a factor of about four times above that observed for the UV-insensitive strains, which in the system used, is definitely significant. In both sensitive strains the supersuppressor rates are approximately equal, but the locus rate is apparently two and a half times greater in XV423-1A. This large increase, however, can probably be explained by the fact that, since XV423-1A has only lys1-1 and ade2-1 as supersuppressible markers (Table 4), some supersuppressor (suppressor 11) mutations, which do not suppress ade2-1 and consequently may turn red, might have been scored as locus revertants. This would not be true with the strain XV423-2A because it carries the supersuppressible trp5-48 marker and suppressor 11 mutations, which do suppress trp5-48, would thus easily be detected. This explanation seems reasonable in view of the observation that many of the supersuppressor revertants, picked onto -LYS and replica plated, turned out to grow very weakly on -ADE until about the fifth day of incubation. Thus, the locus reversion rate in rad3-12 allele-bearing strains is probably more of the order of 2×10^{-8} to 3×10^{-8} mutational events per cell per generation.

The kinetics for lysine revertant appearance for all the strains

discussed above are given in Figures 9 to 12.

Effects of Radiosensitivity Alleles Involved in the Excision Repair Pathway on Reversion Rates to Histidine Independence. Spontaneous reversion rates to histidine independence in strains carrying a mutant allele of one of the rad loci involved in the excision repair pathway are indicated in Table 7. Examination of histidine reversion rates for UV-insensitive strains shows that these are highly variable. Figure 13 illustrates that the reversion rates to histidine independence vary from about 6×10^{-8} to as much as 11×10^{-8} mutational events per cell per generation for the UV-insensitive strains used in these experiments. Histidine reversion rates of up to 14×10^{-8} mutational events per cell per generation have been observed in non-mutator control strains in other experiments (von Borstel, unpublished data). Several factors can account for the wide fluctuation observed in histidine reversion rates. A comparison of Figures 9 through 12 with Figures 14 through 17 illustrates that the number of lysine revertants in all the strains tested has reached a definite plateau level by the tenth day of incubation, if not earlier, while the number of histidine revertants rarely shows such a plateau, even in UV-insensitive strains. Rather, there seems to be a continuous slight increase in the number of histidine revertants, the amount varying with each strain. Because of this effect, scoring histidine reversion experiments at 12 days is an arbitrary choice and some variation between strains is possible. Another factor affecting histidine experiments includes the presence of several pale revertants which when picked onto -HIS do not generally grow. It is difficult to say

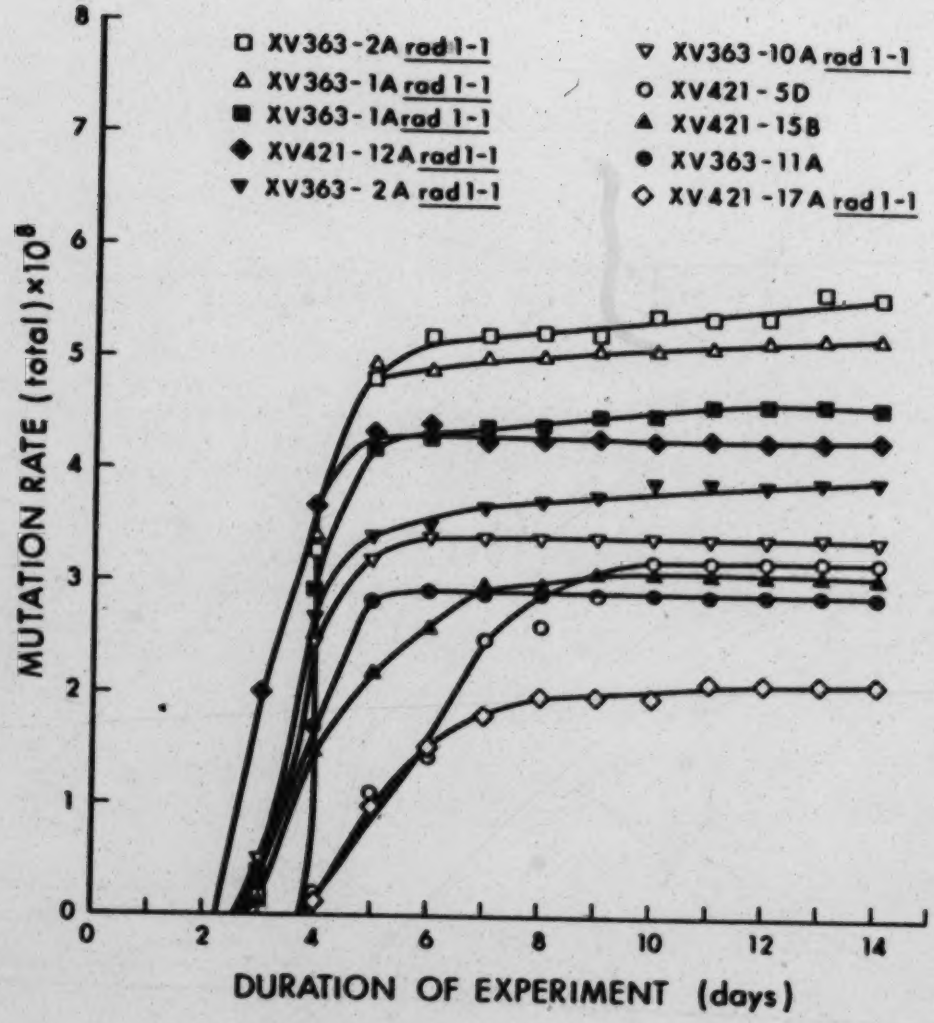


Figure 9. Kinetics of lysine revertant appearance for rad1-1 allele-bearing strains.

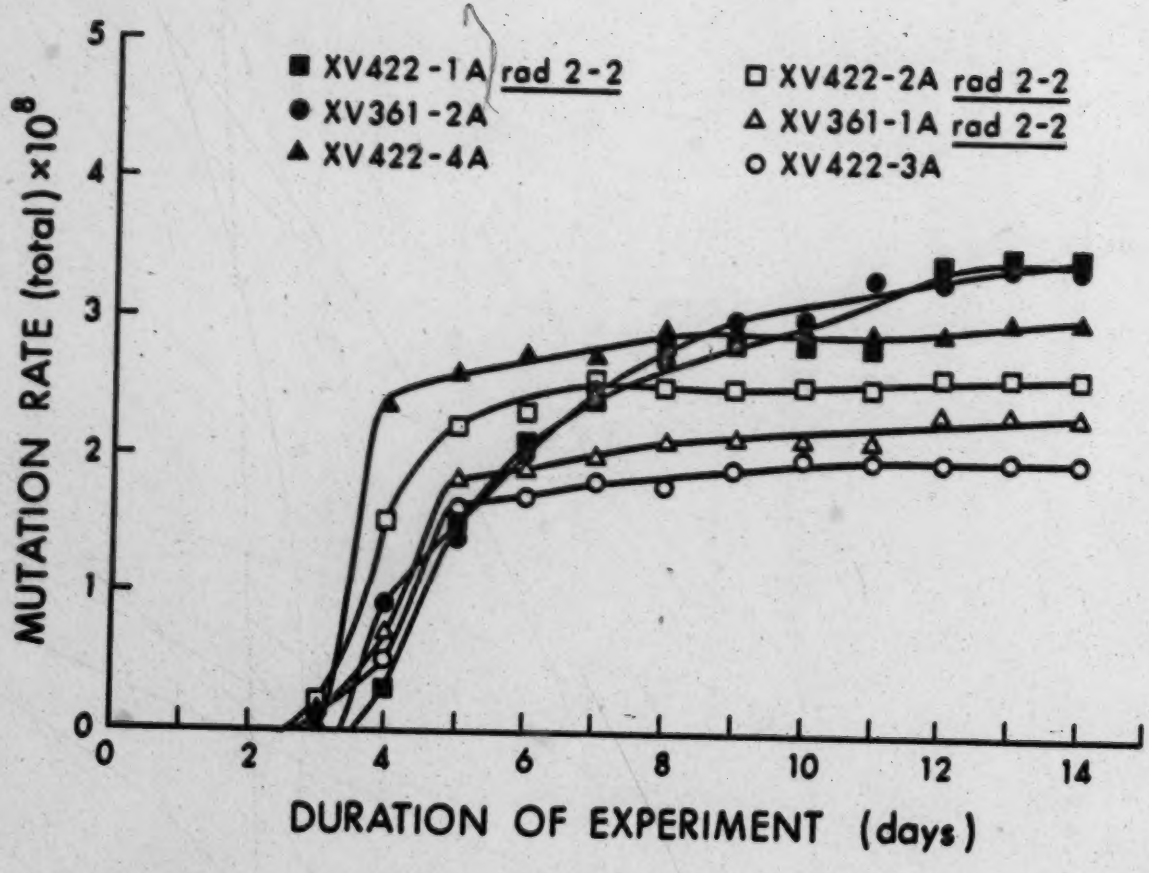


Figure 10. Kinetics of lysine revertant appearance for rad2-2 allele-bearing strains.

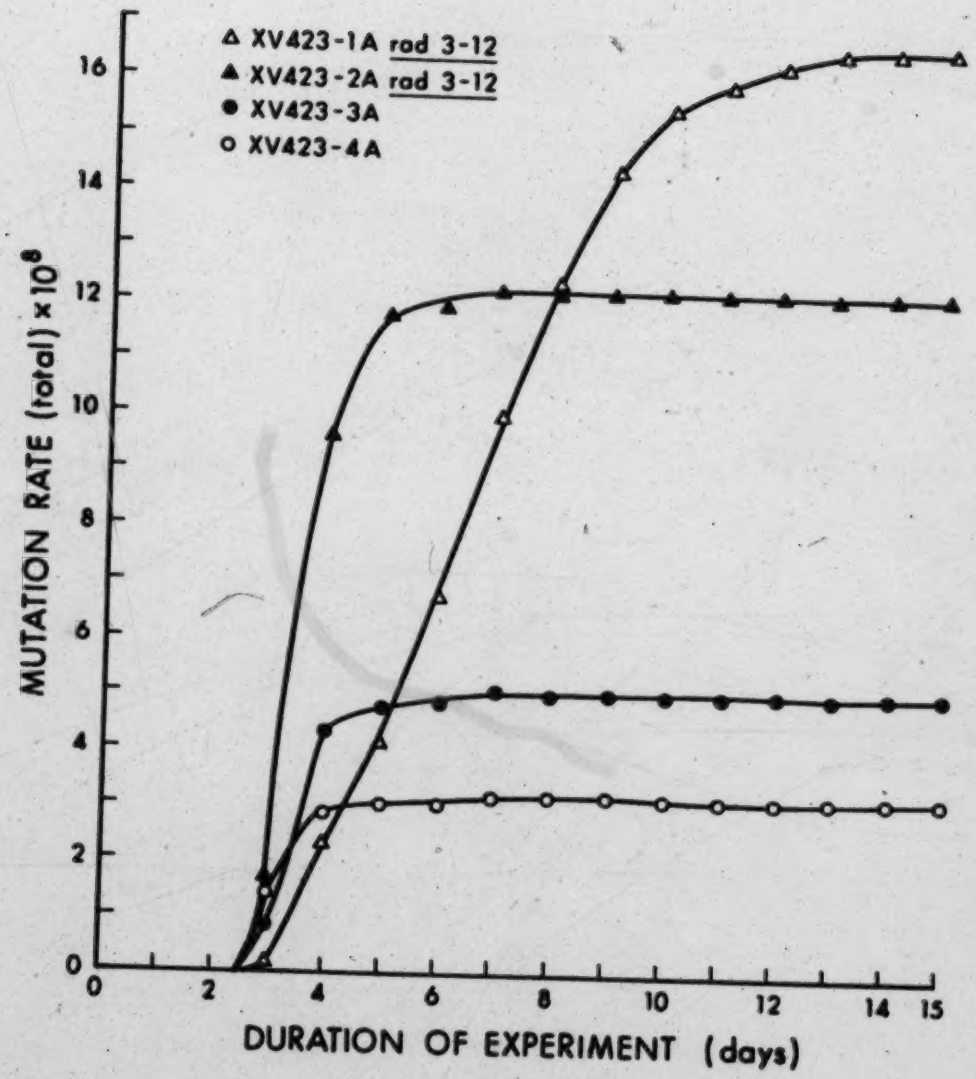


Figure 11. Kinetics of lysine revertant appearance for rad3-12 allele-bearing strains.

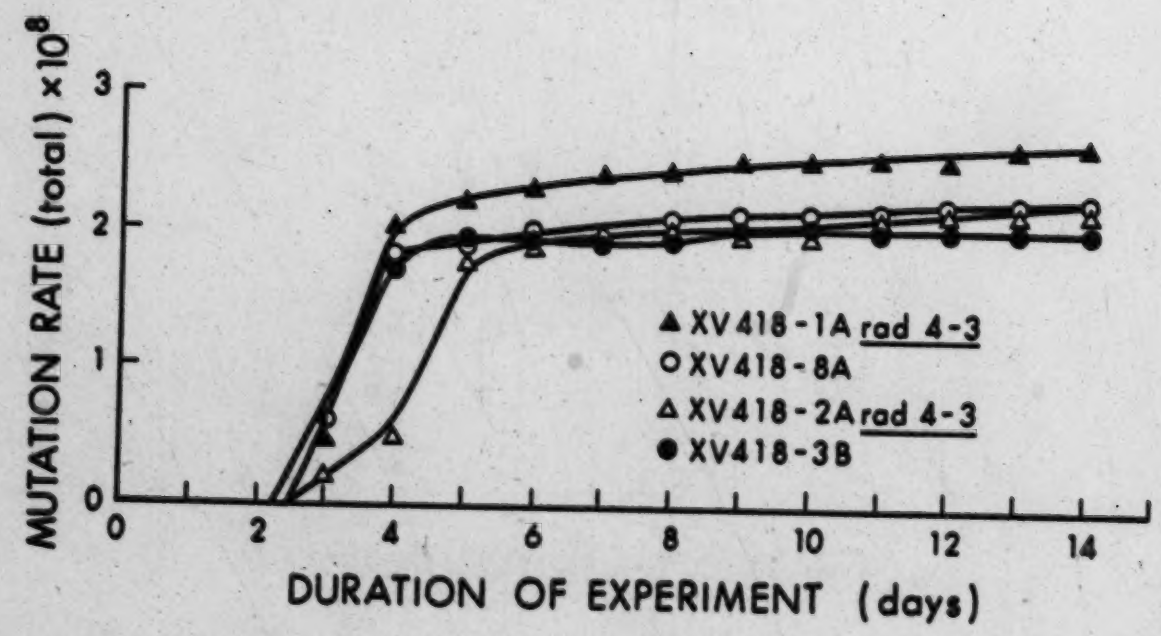


Figure 12. Kinetics of lysine revertant appearance for rad4-3 allele-bearing strains.

TABLE 7. Spontaneous reversion rates to histidine independence in haploid strains bearing a rad or its wild type allele involved in the excision repair pathway

Expt. No.	Strain	rad Allele	Cells/ml (x 10^{-6})	Total Compts	No. of Revertants	Mutation Rate (x 10^{8})
-	XV363-11A		-	-	-	-
50.28	XV421-5D		1.7	999	265	9.1
50.30	XV421-15B		1.7	1002	314	11.0
	Average:					10.1
47.05	XV363-1A	rad1-1	1.0	990	339	21.1
47.06	XV363-2A	rad1-1	1.3	999	331	15.7
-	XV363-10A	rad1-1	-	-	-	-
50.16	XV421-12A	rad1-1	1.7	1001	242	8.4
50.18	XV421-17A	rad1-1	1.7	997	370	13.4
	Average:					14.7
50.09	XV361-2A		1.6	1010	280	9.8
47.09	XV361-2A		2.4	1000	289	7.0
50.26	XV422-3A		1.8	1000	235	7.3
50.22	XV422-4A		1.4	998	259	10.4
	Average:					8.6
43.34	XV361-1A	rad2-2	1.2	999	286	13.7
50.20	XV422-1A	rad2-2	1.8	894	272	10.0
50.24	XV422-2A	rad2-2	1.8	1003	308	10.4
	Average:					11.4
50.34	XV423-3A		1.5	1000	274	10.8
50.36	XV423-4A		1.7	996	208	6.7
	Average:					8.8
50.32	XV423-1A	rad3-12	1.6	1005	1000	166.5
50.38	XV423-2A	rad3-12	1.1	999	649	47.8
	Average:					107.2
47.19	XV418-3B		1.6	1000	194	6.9
47.18	XV418-8A		1.7	898	168	6.1
	Average:					6.5
47.16	XV418-1A	rad4-3	1.6	997	345	13.5
47.17	XV418-2A	rad4-3	1.7	1001	352	12.8
	Average:					13.2

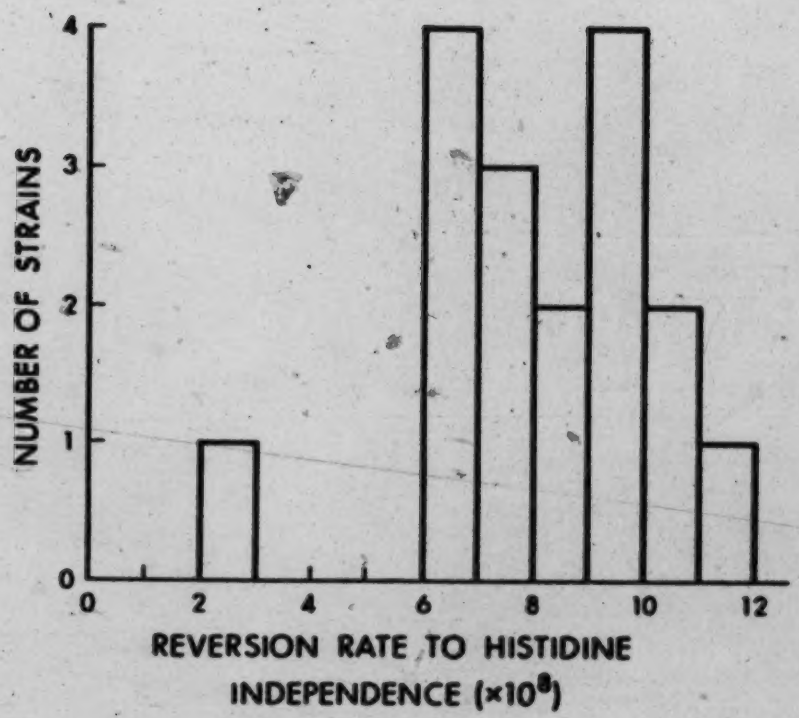


Figure 13. Frequency distribution of the spontaneous reversion rates to histidine independence in strains insensitive to ultraviolet radiation.

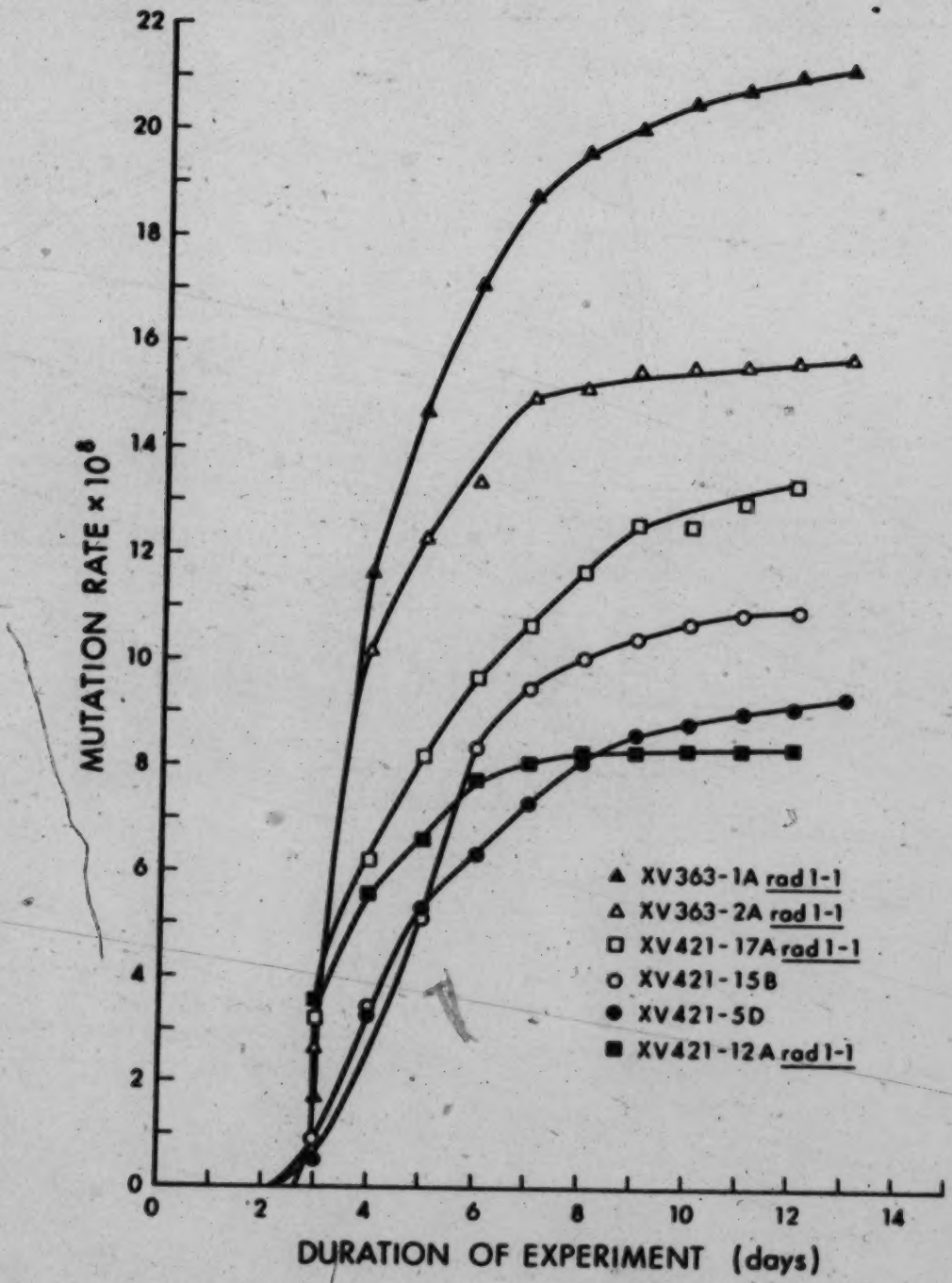


Figure 14. Kinetics of histidine revertant appearance for rad1-1 allele-bearing strains.

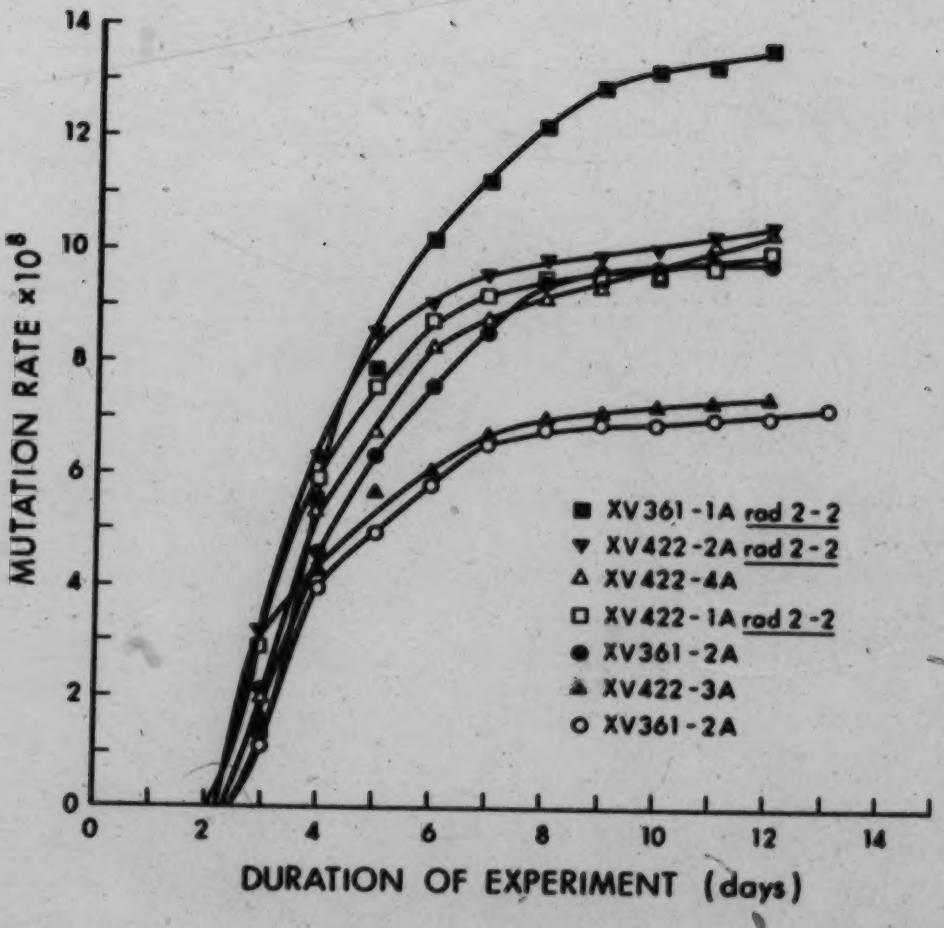


Figure 15. Kinetics of histidine revertant appearance for rad2-2 allele-bearing strains.

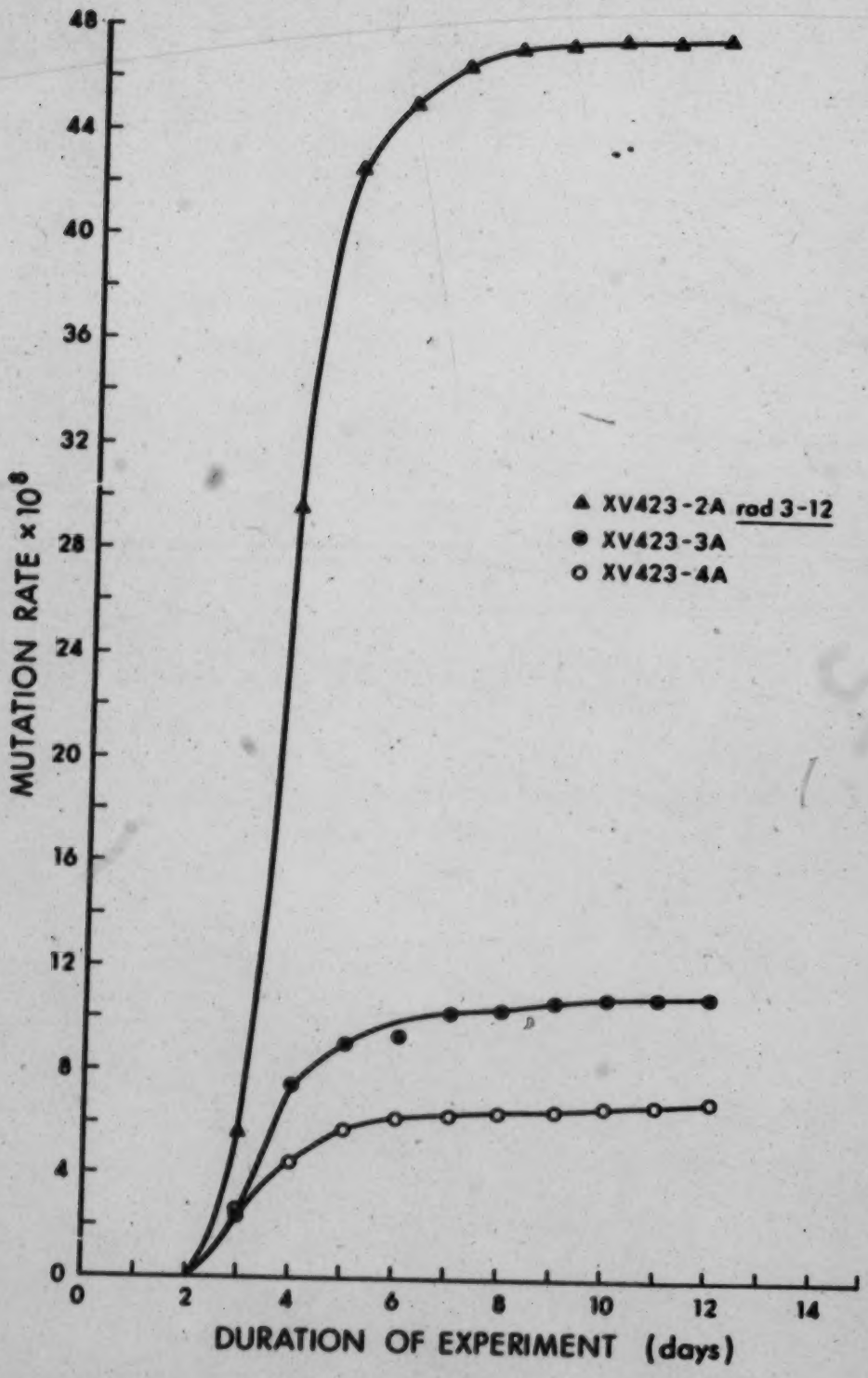


Figure 16. Kinetics of histidine revertant appearance for rad3-12 allele-bearing strains.

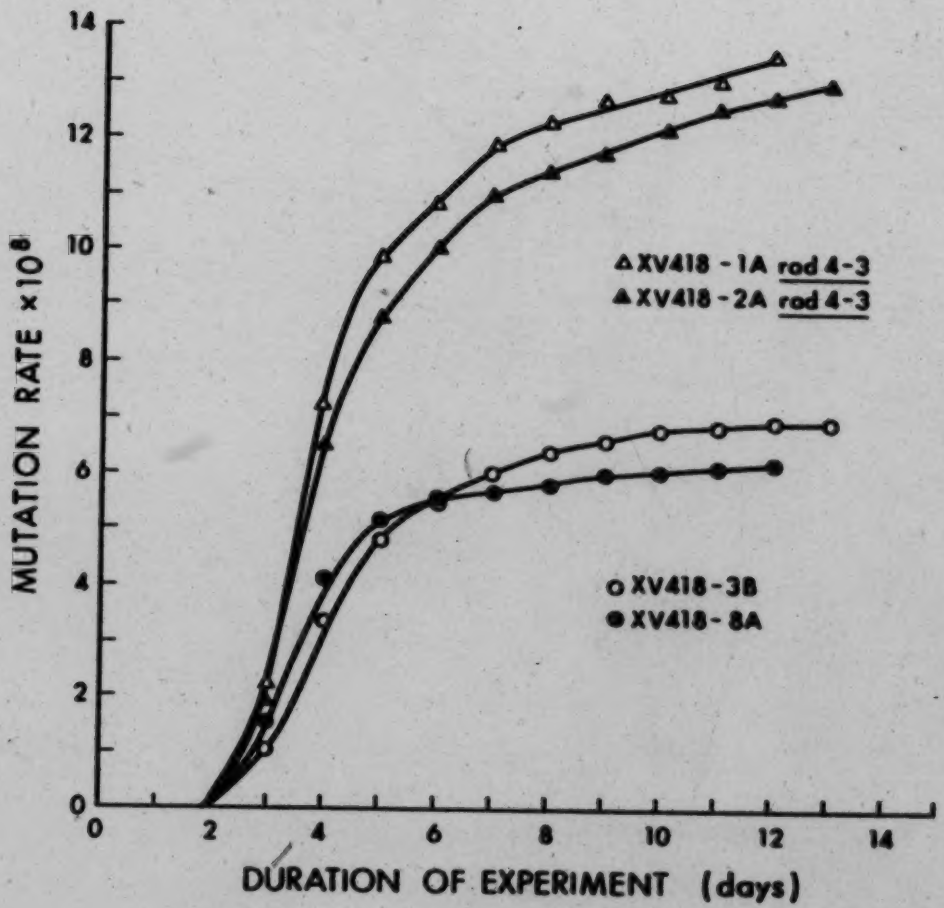


Figure 17. Kinetics of histidine revertant appearance for rad4-3 allele-bearing strains.

if these are really histidine revertants. In general, histidine revertants picked onto -HIS do not grow as well as lysine revertants and consequently replica plating results are more difficult to score. Thus, this method for measuring the spontaneous mutation rate is less accurate for histidine than for lysine experiments.

Table 7 indicates that the UV-sensitive rad1-1 strains obtained from the two separate crosses give seemingly contradictory results. The strains derived from the XV363 diploid show an apparent increase in reversion rate to histidine independence, while strains derived from the XV421 diploid display a reversion rate within the normal range observed for UV-insensitive strains. The increased reversion rate found in XV363-1A and XV363-2A strains may be due partly to the great variation observed for histidine reversion rates in general and partly to the variability to which mutation rates in these particular strains seem to be subject. Therefore, the rad1-1 allele most likely does not confer a significant increase in histidine reversion rate either. Average rates for UV-insensitive and UV-sensitive strains should probably be considered as the true values and these do not differ greatly.

Because of the variation observed in different single colony isolates of one UV-insensitive strain (XV361-2A) with respect to histidine reversion rate, the variation between rad2-2 and UV-insensitive strains within each cross and between rad2-2 strains from different crosses is not considered significant. For rad4-3 -derived strains, however, there is an apparent doubling of the spontaneous histidine reversion rate in the UV-sensitive as compared to the UV-insensitive

strains. Nevertheless, due to the high variation observed in histidine reversion rates, the possibility exists that, if more UV-insensitive strains had been studied, higher values might have been found. Thus, since values for the UV-insensitive strains represent the lowest part of the observed range and the UV-sensitive strain values fall within the range observed in other strains and are not much greater than the rates found in UV-sensitive strains, it seems very likely that rad4-3 also does not confer any significant increase in histidine reversion rate.

The UV-insensitive strains derived from the rad3-12 cross again demonstrate that a fairly high variation can exist even among UV-insensitive strains. Nevertheless the radiosensitivity allele rad3-12 unmistakably confers an average thirteenfold increase in histidine reversion rate. However, the value for XV423-1A, it should be noted, is subject to error because almost all the compartments possessed several revertants in them and cell counts had to be based solely on three compartments without revertants.

The kinetics for histidine revertant appearance for these strains are shown in Figures 14 to 17. As stated above, the reversion rate to histidine independence does not always reach a definite plateau by twelve days; however, the increase in the number of revertants after about ten days is only slight.

Effects of Radiosensitivity Alleles Involved in Other Repair Pathways on Reversion Rates to Lysine Independence. The loci RAD5, RAD8, RAD10, and RAD18 do not control steps in excision repair. For the most part, the repair pathways in which they are involved are

unknown or only speculated upon. The lysine reversion rates for strains carrying a mutant allele of one of these loci are given in Table 8. The genotypes of the strains used are given in Table 5. The average total reversion rate of all the UV-insensitive strains derived from crosses involving these alleles is 2.5×10^{-8} mutational events per cell per generation (average locus reversion rate is 0.3×10^{-8} mutational events per cell per generation; average supersuppressor reversion rate is 2.2×10^{-8} mutational events per cell per generation).

From Table 8, the rad5-1 allele is seen to increase the spontaneous reversion rate to lysine independence by an average factor of eight times above that observed in RAD5-1 strains and there is obviously a fairly large range of variability in its effect. There is a definite increase in mutation rate for the supersuppressor mutations, but the increase in locus rate is more questionable. Once again the higher locus rate observed in XV419-5A may be due to classification of suppressor II mutations as locus revertants, because this strain lacks the supersuppressible tryptophan and arginine markers (Table 5).

The situation presented by the rad8-1 allele is directly opposite to that of rad5-1. The rates of lysine reversion in the UV-insensitive strains are average and within the expected range, whereas those of the rad8-1 allele-bearing strains are decreased below the usually-observed range. Some error due to the low cell count may be involved but the apparent effect of rad8-1 seems to be that of decreasing the mutation rate. Because of the low numbers of locus

TABLE 8. Spontaneous reversion rates to lysine independence in haploid strains bearing a rad or its wild type allele involved in unknown repair pathways

Expt. No.	Strain	rad Allele	Cells/ml (x 10^{-6})	Total Compts.	No. of Revertants Locus	No. of Revertants SS	Mutation Rate ($\times 10^{8}$) Total	Mutation Rate ($\times 10^{8}$) Locus	Mutation Rate ($\times 10^{8}$) SS
47.22	XV419-11A		1.7	998	6	56	1.9	0.2	1.7
47.23	XV419-12A		1.9	1001	9	79	2.4	0.2	2.2
	Average:						2.2	0.2	2.0
47.15	XV419-2A	rad5-1	1.8	999	15	378	13.6	0.4	13.2
47.21	XV419-5A	rad5-1	1.7	1002	40	495	21.2	1.2	20.0
50.04	XV419-13A	rad5-1	1.7	1001	27	400	15.8	0.8	15.0
	Average:						16.7	0.6	16.1
47.26	XV420-4A		1.1	988	9	73	3.7	0.4	3.3
50.01	XV420-6A		2.1	998	8	108	3.0	0.2	2.8
	Average:						3.4	0.3	3.1
47.24	XV420-1A	rad8-1	1.1	995	3	14	0.8	0.1	0.7
47.25	XV420-2A	rad8-1	0.8	999	2	17	1.1	0.1	1.0
50.03	XV420-3A	rad8-1	2.3	992	25	26	1.2	0.6	0.6
	Average:						1.1	0.3	0.8
43.22	XV366-2A		1.8	988	15	64	2.3	0.4	1.9
43.27	XV366-4A		1.6	996	2	33	1.2	0.1	1.1
43.20	XV366-5A		1.6	997	11	86	2.1	0.3	1.8
	Average:						1.9	0.3	1.6
43.21	XV366-3A	rad10-1	1.5	997	3	52	1.8	0.1	1.7
43.26	XV366-6A	rad10-1	1.7	995	5	39	1.4	0.2	1.2
43.30	XV365-2A	rad10-1	1.5	999	40	92	4.6	1.4	3.2
43.28	XV365-4A	rad10-1	1.2	999	15	42	2.6	0.7	1.9
	Average:						2.6	0.6	2.0
43.04*	XV362-3A		2.0	995	28	11	1.0	0.7	0.3
43.03*	XV362-5A	rad18-1	1.2	990	10	112	5.5	0.4	5.1
43.05*	XV362-5A	rad18-1	1.3	1000	8	154	6.8	0.3	6.5
43.02*	XV362-6A	rad18-1	1.7	998	5	20	0.8	0.2	0.6
43.06*	XV362-6A	rad18-1	1.7	996	5	24	0.7	0.1	0.7
43.07*	KC372	rad18-2	1.1	998	11	265	13.8	0.3	13.5

* Mutation rates for rad18-1 were calculated from the number of revertants present on day 14.

revertants it is difficult to conclude definitely regarding its effect on locus reversions, but in these strains bearing the rad8-1 allele there is at least a decrease in the supersuppressor reversion rate.

The UV-insensitive RAD10-1 strains have reversion rates within the lower part of the range observed for UV-insensitive strains in general, this being true especially for XV366-4A. There is not much difference between the two rad10-1-carrying strains derived from the XV366 diploid, both of which fall within the UV-insensitive strain range. The reversion rates of two other strains, sisters of the radiation-sensitive parent of the XV366 diploid, were also measured. XV365-4A demonstrates a normal reversion rate to lysine independence while XV365-2A seems to show a slight increase, though it is still within the UV-insensitive strain range. The latter strain was also observed to behave peculiarly when the box revertants picked for analysis were replica plated. The locus revertants were all found to remain red on YEPD even though they were overgrown with secondary supersuppressor mutations on -ADE and -ARG plates and consequently should have shown the white overgrowth characteristic of such mutations on YEPD. Thus, the possibility of two adenine mutants, one suppressible and the other not, cannot be eliminated. This could theoretically serve to increase the locus rate to the high value observed.

The behaviour of the rad18 locus represents a complicated situation. The first allele of this locus to be studied was rad18-2. Its average reversion rate to lysine independence was found to be 24.3 x

10^{-8} mutational events per cell per generation (von Borstel et al., 1971). Another culture of the same strain was here retested and the mutation rate was found to be lower (Table 8), though still approximately four to five times greater than the UV-insensitive strain values. An attempt was then made to measure the reversion rate of its allele rad18-1 to determine if it too conferred an increased reversion rate to lysine independence. The results shown in Table 8 illustrate that the two rad18-1 allele-bearing strains tested gave contradictory results. XV362-5A apparently causes perhaps a slight increase in mutation rate, over and above the UV-insensitive strain range, though it seems not to be as powerful a mutator as rad18-2. Meanwhile, XV362-6A seems to decrease the supersuppressor mutation rate drastically. Repetition of the fluctuation test on a different single colony isolate of the same strain in each case reconfirmed the original finding. The RAD18-1 strain derived from the XV362 diploid was also found to behave strangely by strongly depressing the supersuppressor reversion rate. It should be noted that for rad18-1 allele-bearing strains, the mutation rates and numbers of revertants given in Table 8 represent those obtained at 14 days' incubation, as the numbers of revertants were still found to increase considerably after day 10.

Figures 18 to 21 show the kinetics of lysine revertant appearance for these strains.

Effects of Radiosensitivity Alleles Involved in Other Repair Pathways on Reversion Rates to Histidine Independence. Spontaneous reversion rates to histidine independence are presented in Table 9.

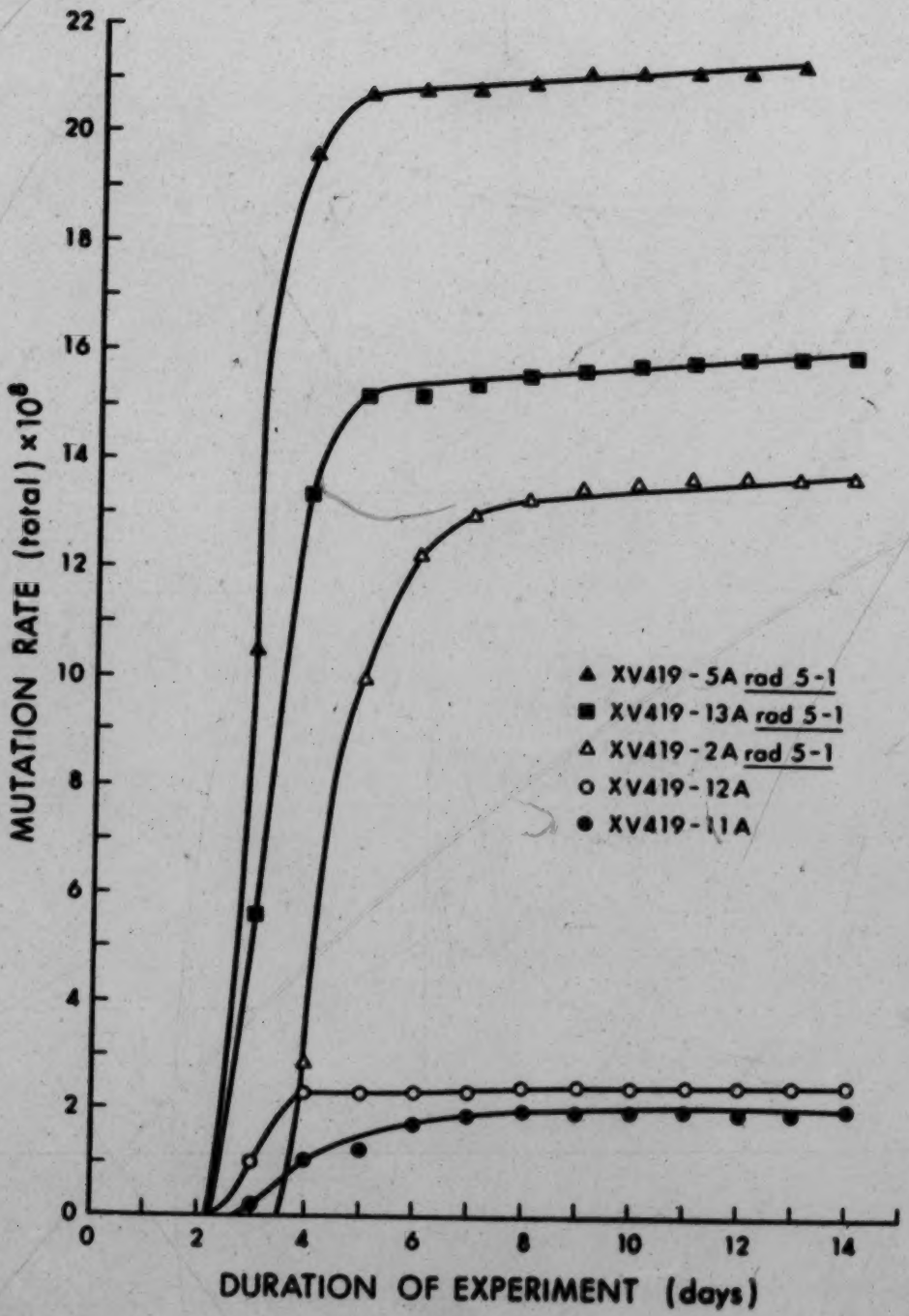


Figure 18. Kinetics of lysine revertant appearance for rad5-1 allele-bearing strains.

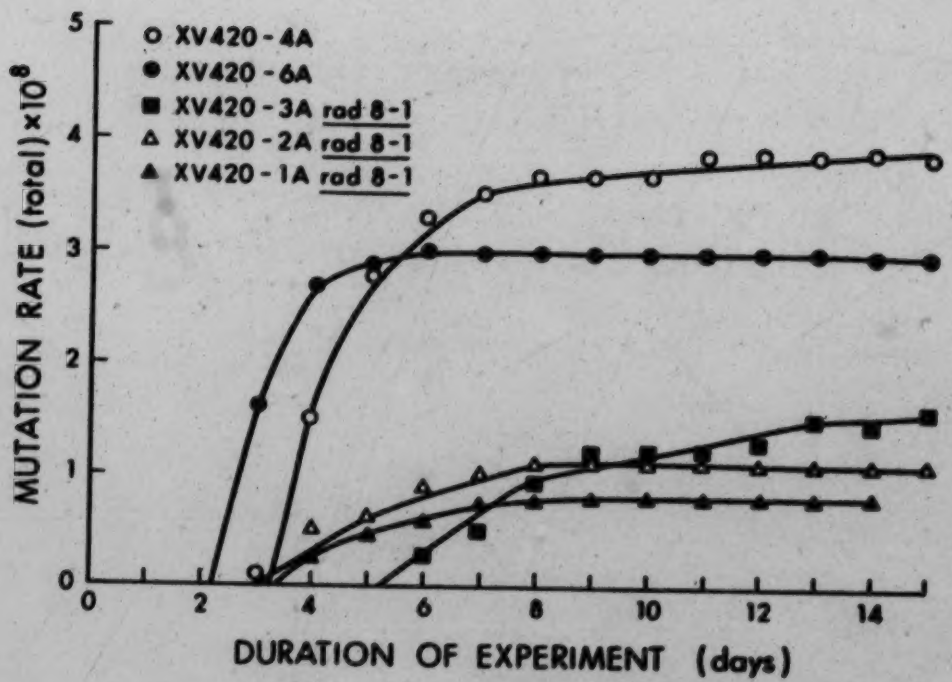


Figure 19. Kinetics of lysine revertant appearance for rad8-1 allele-bearing strains.

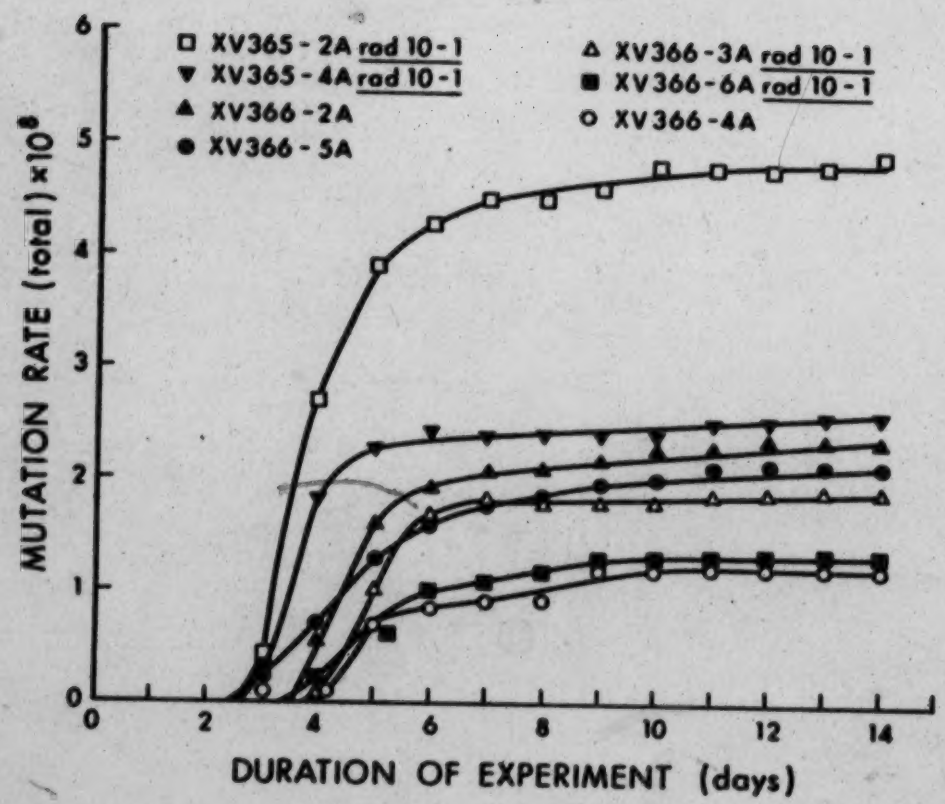


Figure 20. Kinetics of lysine revertant appearance for rad10-1 allele-bearing strains.

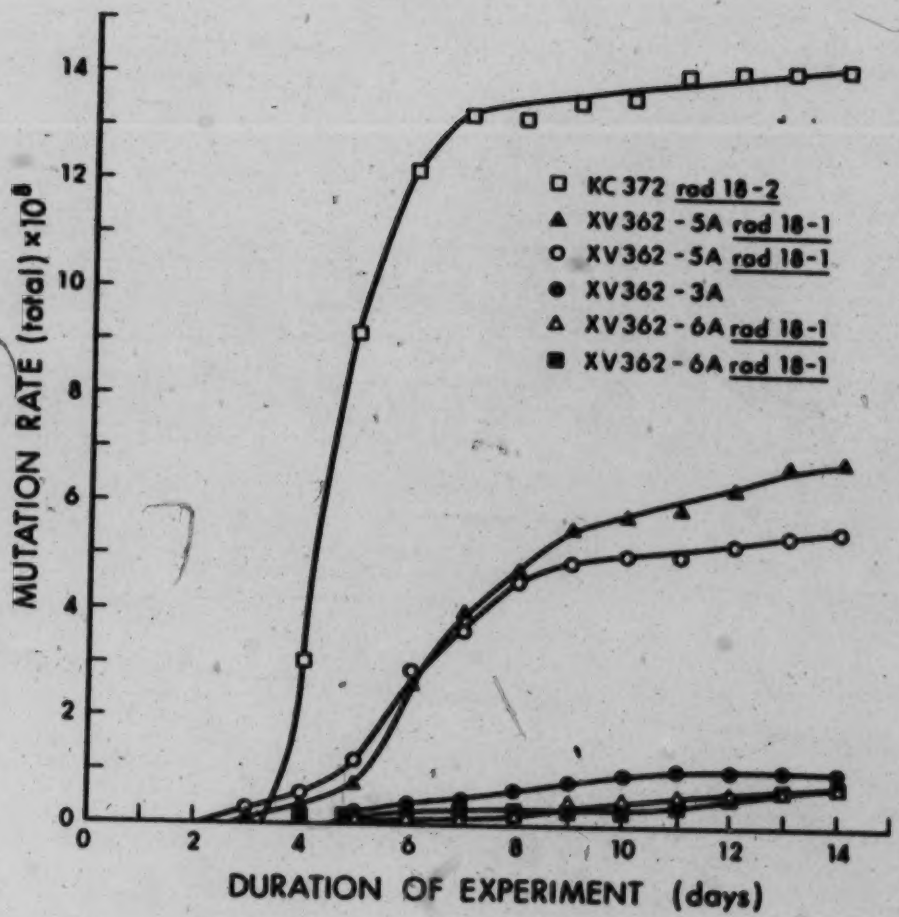


Figure 21. Kinetics of lysine revertant appearance for rad18-1 and rad18-2 allele-bearing strains.

TABLE 9. Spontaneous reversion rates to histidine independence in haploid strains bearing a rad or its wild type allele involved in unknown repair pathways

Expt. No.	Strain	rad Allele	Cells/ml (x 10^{-6})	Total Compts.	No. of Revertants	Mutation Rate (x 10^{-8})
50.12	XV419-11A		1.6	999	251	9.2
50.13	XV419-12A		1.4	999	190	7.5
	Average:					8.4
47.20	XV419-2A	rad5-1	1.8	1000	625	27.1
50.11	XV419-5A	rad5-1	1.4	1000	663	36.6
50.10	XV419-13A	rad5-1	1.6	999	480	20.3
	Average:					26.0
50.07	XV420-4A		1.1	1000	141	6.8
50.08	XV420-6A		1.7	1000	97	2.9
	Average:					4.9
50.14	XV420-1A	rad8-1	1.0	999	78	4.2
50.06	XV420-2A	rad8-1	0.7	1000	24	1.8
-	XV420-3A	rad8-1	-	-	-	-
	Average:					3.0
43.25	XV366-2A		1.7	997	240	8.2
43.32	XV366-4A		1.3	998	199	8.3
43.23	XV366-5A		1.4	997	246	9.6
	Average:					8.7
43.24	XV366-3A	rad10-1	1.5	1001	252	9.1
43.31	XV366-6A	rad10-1	1.4	997	297	12.7
50.05	XV365-2A	rad10-1	1.8	999	466	17.1
47.10	XV365-2A	rad10-1	1.7	997	521	21.4
43.33	XV365-4A	rad10-1	1.2	999	248	11.7
	Average:					14.4
-	XV362-3A		-	-	-	-
-	XV362-5A	rad18-1	-	-	-	-
-	XV362-6A	rad18-1	-	-	-	-
-	KC372	rad18-2	-	-	-	-

The radiation-sensitive strains bearing the rad5-1 allele show an increased rate of reversion to histidine prototrophy. As with lysine, there is some variation observed but the increase seems to be by an average factor of three. The effect is real as it is shown by all three UV-sensitive strains tested and cannot be ascribed to chance variation.

The decrease in reversion rate observed for lysine in rad8-1 strains seems to hold also for histidine. However, it is unusual that the UV-insensitive strain XV420-6A should show a decreased reversion rate, even lower than one of the UV-sensitive rad8-1 strains.

Both of the two UV-sensitive rad10-1 strains, derived from the XV366 diploid, show reversion rates within the UV-insensitive strain range. Of the two strains derived from the XV365 diploid, the one which showed the increase in lysine reversion rate also shows an increased histidine reversion rate. However, because none of the other rad10-1 strains showed a similar effect, it can probably be concluded that this increase is not due to any effect of the rad10-1 allele but rather is the result of a weak background mutator or simply due to the fluctuation and error observed in histidine experiments.

Kinetics of histidine revertant appearance are given in Figures 22, 23, and 24 for strains bearing rad5-1, rad8-1, and rad10-1 radiosensitivity alleles. Histidine reversion studies on rad18-1 and rad18-2 were not performed.

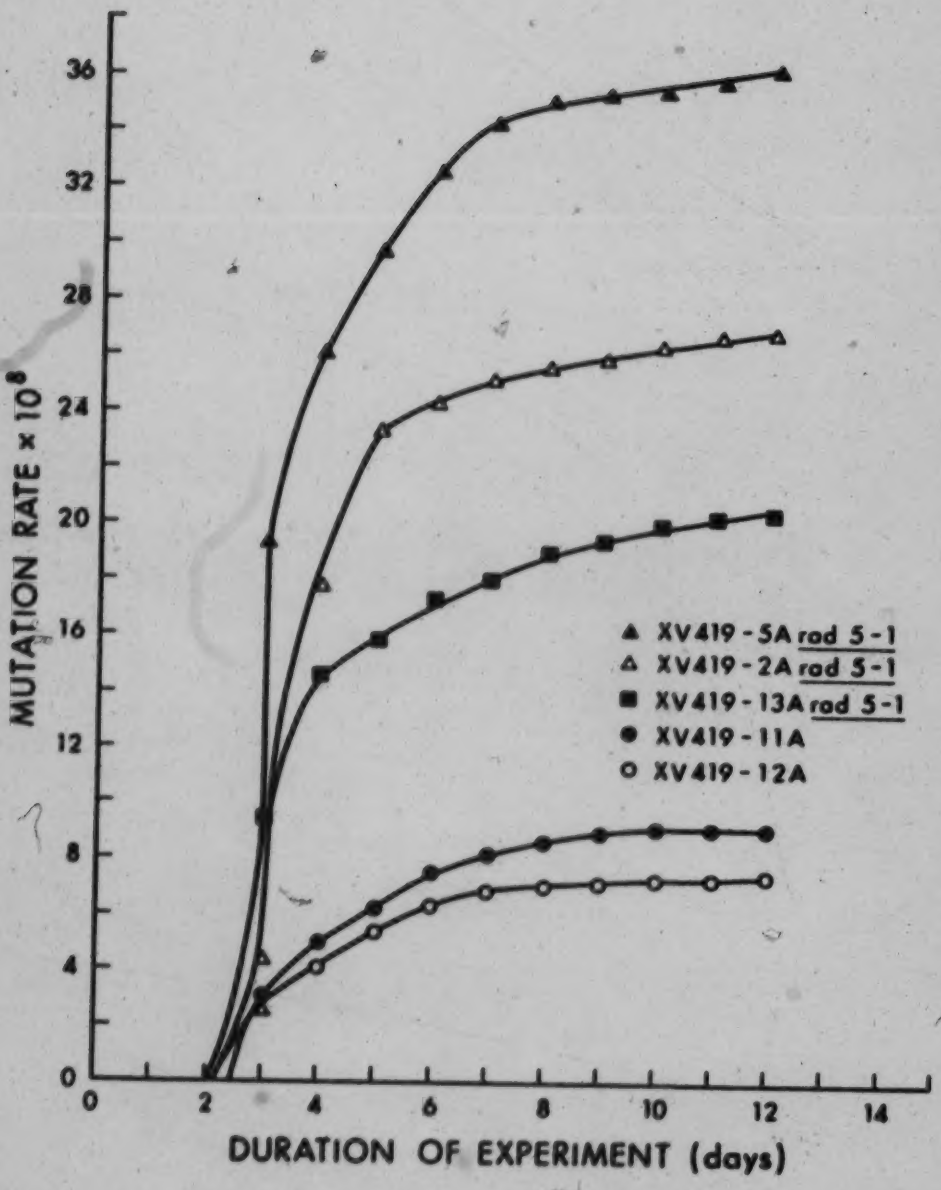


Figure 22. Kinetics of histidine revertant appearance for rad5-1 allele-bearing strains.

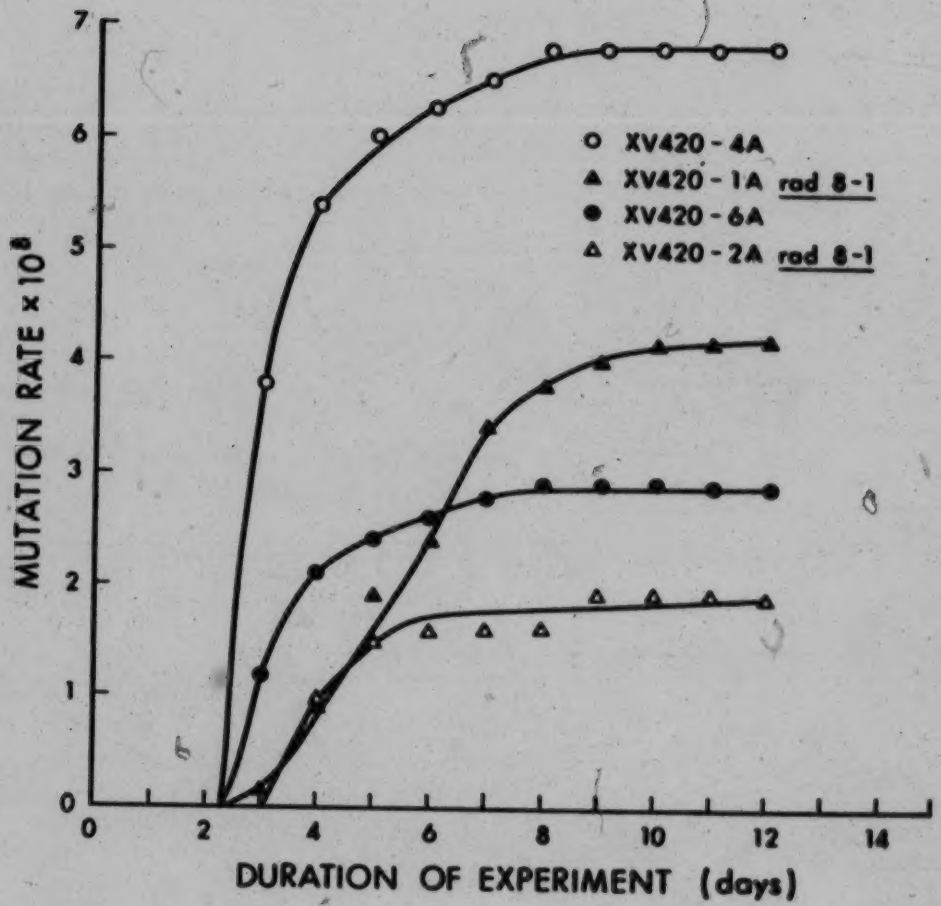


Figure 23. Kinetics of histidine revertant appearance for rad8-1 allele-bearing strains.

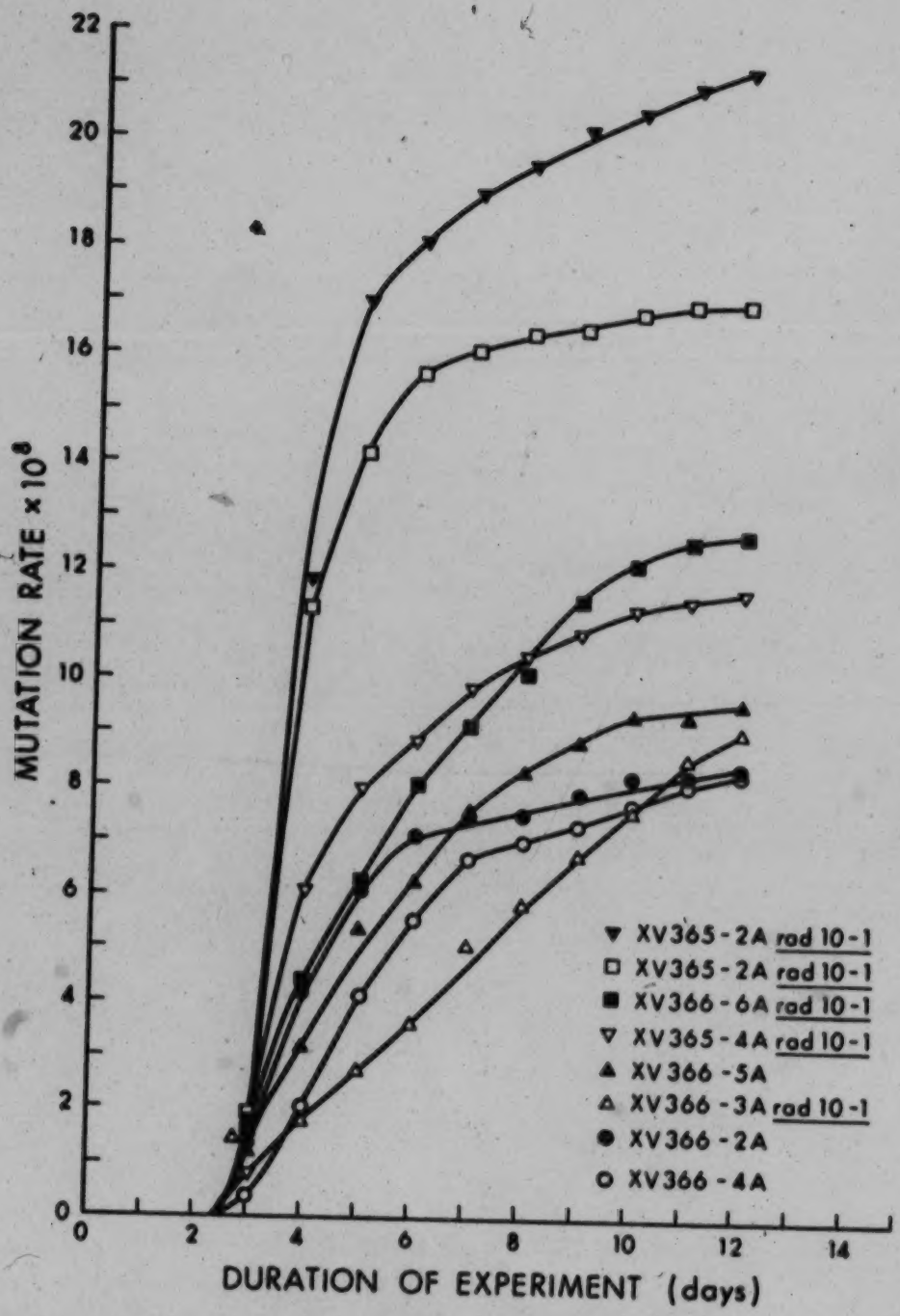


Figure 24. Kinetics of histidine revertant appearance for rad10-1 allele-bearing strains.

A Qualitative Estimation of Mutator Activity

The "Lassie" Test. Because the accurate measurement of spontaneous mutation rates by means of the 1000-compartment fluctuation experiment is a long and tedious process and could only be done on a few strains from each cross, a "lassie" test was carried out by plating as many sister strains as possible on MC media. After 10 days' incubation a low colony count on MC, generally less than 50 revertants per plate, indicates a normal spontaneous mutation rate to lysine independence; a high count represents a mutator. The average colony count on MC for a non-mutator strain such as XV185-14C is between 10 to 25 colonies per plate (von Borstel, unpublished data). On the average, non-mutator strains derived from crosses of each of the rad strains gave between 20 to 30 revertant colonies per plate, though occasionally more than 30 were found. High mutators, on the other hand, generally give more than 100 colonies per plate, though sometimes less than 100 were counted.

"Lassie" tests can be quite accurate with non-mutator strains, provided the cell concentration is within certain limits (1×10^6 to 9×10^6 cells/ml) and the thickness of the media does not vary greatly (von Borstel, unpublished data). Although it is possible that some of the variation observed between plates could be due to unequal thickness of the culture media, examination of UV-insensitive strains indicates that genetic background variation must also play an important role.

Although it is basically a qualitative rather than a quantitative estimation of mutation rate, an attempt was made to standardize

the "lassie" test, that is to correlate the number of colonies per MC plate with the mutation rate calculated on the basis of the fluctuation test. From data based mostly on the rad10-1 cross, wherein several plates of each strain were scored, it is possible to roughly equate 10 colonies on an MC plate with a mutation rate of 1×10^{-8} mutational events per cell per generation. Thus 20 colonies represents approximately a mutation rate of 2×10^{-8} mutational events per cell per generation, 30 colonies a mutation rate of 3×10^{-8} mutational events per cell per generation, etc. This correlation has been found to predict within limits most of the average reversion rates to lysine independence quite satisfactorily, except for mutators which show less than 100 colonies per MC plate. It should be noted, however, that the "lassie" test can be used only to predict mutator activity with respect to lysine reversion. No similar satisfactory test for predicting histidine reversion rates has yet been devised.

The Effects of Radiosensitivity Alleles on the Number of Revertant Colonies per MC Plate. The average number of colonies per MC plate and the observed range in colony counts are given in Tables 10 through 20 for all the strains tested. Actual colony counts on each MC plate may be found in the Appendix, Tables A8 to A18. In calculating averages and plotting histograms below, correction for a high average number of background revertants was made by subtracting this number from the average MC colony count.

Table 10 gives the average number of colonies per MC plate for strains derived from the two crosses involving the rad1-1 allele. The frequency distributions of the average numbers of colonies per MC

TABLE 10. Number of colonies on MC plates shown by the UV-sensitive and UV-insensitive haploid strains derived from the crosses of XS774-5D radl-1 x XV169-12A and XS774-5D radl-1 x XV185-6A

Strain	UV Sensitivity	Average No. of Background Revertants	No. of Plates	Average No. of Colonies Per MC Plate	Range
*XV363-11A	Insensitive	1	2	31	25-35
XV421-2B		1	1	68	70
XV421-3C		0	1	78	80
*XV421-5D		0	3	35	25-45
XV421-7D		0	2	37	35-40
XV421-9D		1	3	17	15-25
XV421-13D		1	3	32	25-40
*XV421-15B		1	2	22	15-30
XV421-16C		0	2	28	25-30
XV421-18B		0	2	23	20-25
			Average:	37	15-80
*XV363-1A	Sensitive	1	2	41	30-55
+XV363-1A		8	5	47	45-55
+*XV363-1A		0	8	39	30-55
*XV363-2A		13	2	49	45-50
+*XV363-2A		0	7	43	35-50
+XV363-2A		1	5	40	35-50
*XV363-10A		0	2	39	35-45
XV421-4A		0	2	36	30-40
XV421-4C		0	2	49	35-60
XV421-5A		1	2	28	25-30
XV421-6A		1	2	48	40-55
XV421-8C		2	2	66	55-75
*XV421-12A		1	3	49	30-65
*XV421-17A		20	3	39	30-50
			Average:	38	30-65

* The mutation rates of these strains were also measured using the 1000-compartment fluctuation test.

+ Another single colony isolate of the same strain.

plate for the UV-sensitive and UV-insensitive strains are given in Figure 25. The predominant values fall in the range of 20 to 50 colonies per plate, but lower and higher averages are found in both UV-insensitive and UV-sensitive strains. The colony counts for the UV-sensitive strains tend to be slightly higher than for UV-insensitive strains, though the average counts are equal for both. It can be seen from Table 10 that the average number of colonies in two UV-sensitive strains from the same tetrad (XV421-4A and XV421-4C) can differ by more than 10 colonies, while one UV-sensitive and one UV-insensitive strain from the same tetrad (XV421-5A and XV421-5D) differ by less than 10 colonies. Similarly, repeating the "lassie" test on different single colony isolates of the same strain, as was done for XV363-1A and XV363-2A, shows that there may be a difference of 10 in the average number of colonies per MC plate, in agreement with the variations observed in actual mutation rates. In effect, the variation observed in MC colony counts here reflects the observed variation of mutation rates in strains derived from the radl-1 crosses. On the basis of the average number of colonies on MC, one can predict an average mutation rate of 2×10^{-8} to 4×10^{-8} mutational events per cell per generation for UV-insensitive strains and between 3×10^{-8} to 5×10^{-8} mutational events per cell per generation for UV-sensitive strains, with some variation in either direction, and this is actually observed. "Lassie" and mutation rate data, therefore, both imply no significant change, but a good deal of variation, in the spontaneous lysine reversion rate for radl-1 allele-bearing strains.

Table 11 presents "lassie" test data obtained for RAD2-2 and

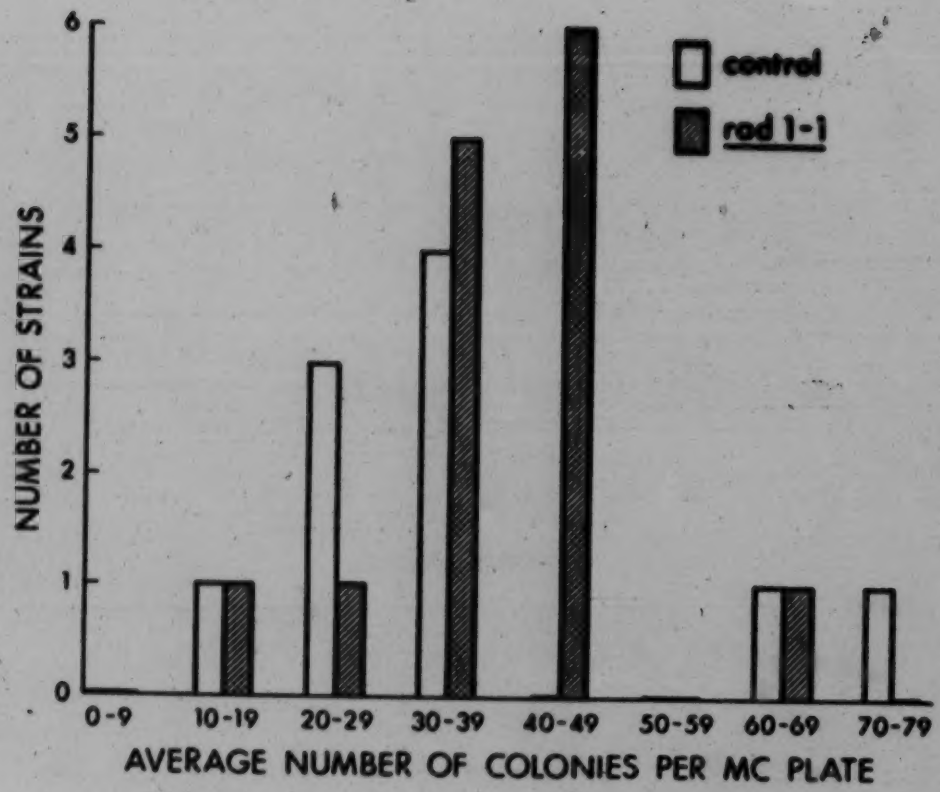


Figure 25. Frequency distributions of the average number of colonies per MC plate in the UV-sensitive and UV-insensitive strains derived from the crosses of XS774-5D rad1-1 x XV169-12A and XS774-5D rad1-1 x XV185-6A.

TABLE 11. Number of colonies on MC plates shown by the UV-sensitive and UV-insensitive haploid strains derived from the crosses of S226-7C rad2-2 x XV169-15A and S226-7C rad2-2 x XV185-14C

Strain	UV Sensitivity	Average No. of Background Revertants	No. of Plates	Average No. of Colonies Per MC Plate	Range
*XV361-2A	Insensitive	1	10	25	15-35
*XV422-3A		0	2	19	15-20
*XV422-4A		1	3	37	30-45
XV422-6A		0	3	25	20-30
XV422-9A		8	3	37	35-40
XV422-10A		1	3	19	15-25
XV422-11A		0	2	16	15
XV422-15A		0	2	19	15-20
			Average:	24	15-35
*XV361-1A	Sensitive	0	2	19	15-20
*XV422-1A		0	2	23	20-25
*XV422-2A		7	2	38	30-45
XV422-5A		0	3	37	30-50
XV422-8A		0	3	19	15-20
XV422-12A		0	2	33	30-35
XV422-13A		2	2	48	30-65
XV422-14A		0	2	40	35-45
			Average:	31	20-50

* The mutation rates of these strains were also measured using the 1000-compartment fluctuation test.

rad2-2 strains. Figure 26 gives the frequency distributions of the average numbers of colonies per MC plate for strains derived from both crosses of the original rad2-2 strain. As with rad1-1, there is a slight tendency toward higher colony counts for rad2-2 strains as compared to the UV-insensitive strains, but average values for both fall within the average UV-insensitive strain range of 20 to 30 colonies. From Table 11, one can once again predict an average mutation rate of about 2×10^{-8} to 3×10^{-8} mutational events per cell per generation for both RAD2-2 and rad2-2 strains and this is what is found.

Table 12 and Figure 27 show that, while the RAD3-12 strain MC colony count values are in the normal non-mutator range, the UV-sensitive rad3-12 strain values clearly tend to be much higher (approximately triple, on the average). This is indicative of an increased reversion rate to lysine independence conferred by the rad3-12 allele, though an exact correlation between colony numbers and mutation rate cannot here be made.

From Table 13 and Figure 28, it can be seen that in rad4-3 strains, as in rad1-1 and rad2-2 strains, the average numbers of colonies per MC plate are slightly higher than in the UV-insensitive strains, but both are within the normal UV-insensitive strain range. One can predict a mutation rate of approximately 2×10^{-8} mutational events per cell per generation for RAD4-3 strains, which is actually the case. The actual mutation rate found in the rad4-3 strains is lower than the predicted average of 3×10^{-8} mutational events per cell per generation, implying that some variation can be expected.

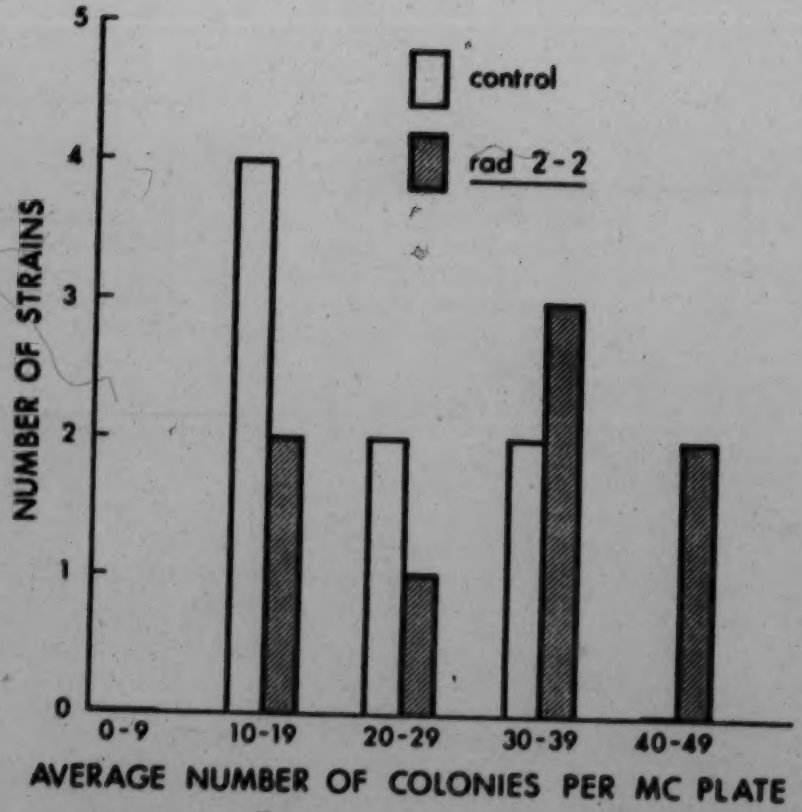


Figure 26. Frequency distributions of the average number of colonies per MC plate in the UV-sensitive and UV-insensitive strains derived from the crosses of S226-7C rad2-2 x XV169-15A and S226-7C rad2-2 x XV185-14C.

TABLE 12. Number of colonies on MC plates shown by the UV-sensitive and UV-insensitive haploid strains derived from the cross of 197/2D rad3-12 x XV185-14C

Strain	UV Sensitivity	Average No. of Background Revertants	No. of Plates	Average No. of Colonies Per MC Plate	Range
*XV423-3A	Insensitive	0	2	40	30-50
*XV423-4A		0	2	17	15-20
XV423-9A		1	2	37	35-40
XV423-10A		0	2	20	15-20
XV423-11A		1	2	20	15-25
XV423-12A		0	2	36	35-40
XV423-13A		0	2	12	10-15
XV423-14A		0	9	21	10-30
XV423-18A		2	2	52	45-55
XV423-20A		1	2	51	45-60
XV423-21A		0	2	36	30-45
XV423-22A		6	2	33	30-35
XV423-25A		0	2	18	15-20
XV423-26A		5	2	38	35-40
			Average:	31	10-50
*XV423-1A	Sensitive	1	2	911	90
*XV423-2A		0	2	69	55-85
+XV423-5A		1	2	162	160-165
XV423-6A		0	2	82	70-95
XV423-7A		2	5	66	45-80
			Average:	94	65-160

* The mutation rates of these strains were also measured using the 1000-compartment fluctuation test.

+ petite strain.

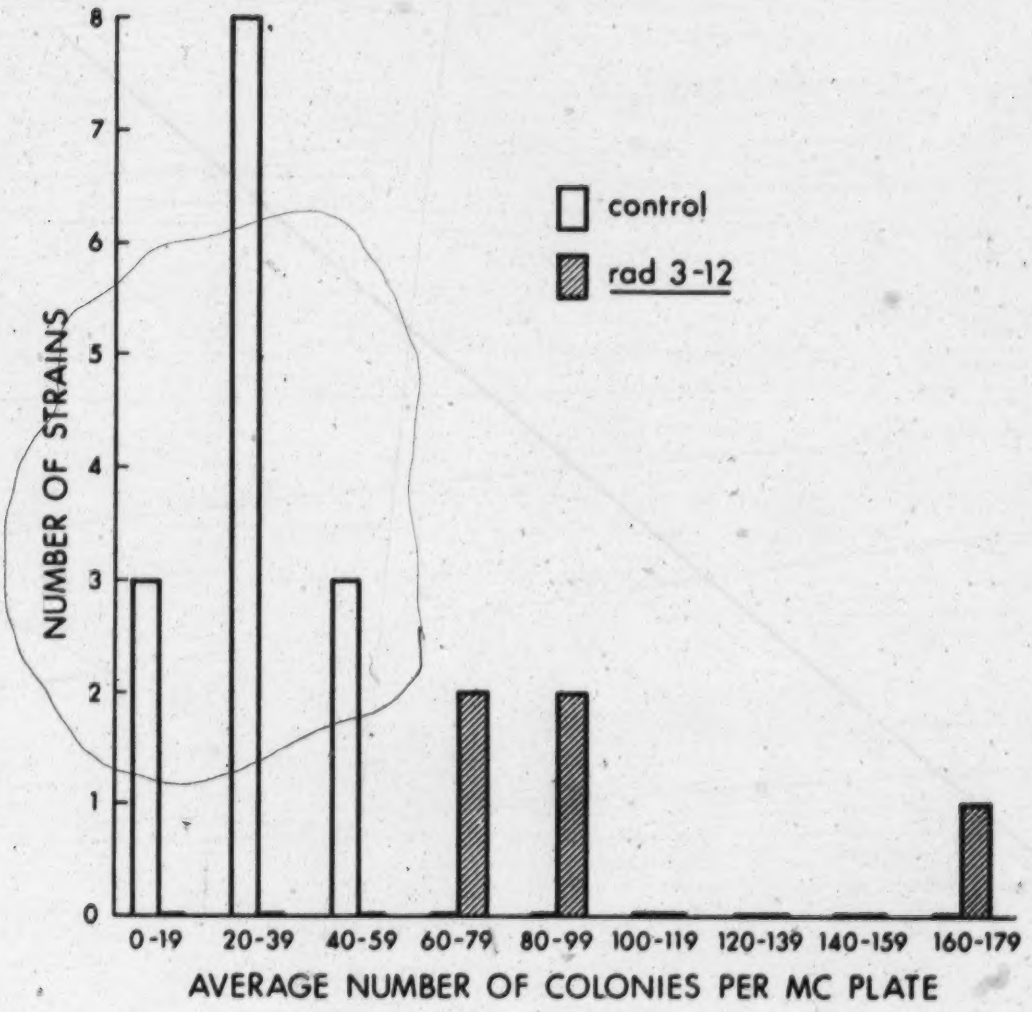


Figure 27. Frequency distributions of the average number of colonies per MC plate in the UV-sensitive and UV-insensitive strains derived from the cross of 197/2D rad3-12 x XV185-14C.

TABLE 13. Number of colonies on MC plates shown by the UV-sensitive and UV-insensitive haploid strains derived from the cross of S960-1A rad4-3 x XV185-14C

Strain	UV Sensitivity	Average No. of Background Revertants	No. of Plates	Average No. of Colonies Per MC Plate	Range
*XV418-8A	Insensitive	1	3	12	10-15
XV418-9A		0	3	11	10-15
*XV418-3B		1	3	29	25-30
XV418-5B		0	3	17	15-20
XV418-6B		1	3	27	20-30
XV418-7B		8	3	29	20-35
			Average:	21	10-35
*XV418-1A	Sensitive	3	3	28	20-35
*XV418-2A		0	3	32	25-35
XV418-3A		18	3	55	45-70
XV418-4A		1	3	36	25-45
XV418-5A		53	3	91	80-105
XV418-6A		1	3	25	20-30
XV418-7A		2	3	46	40-55
XV418-2B		0	3	22	10-30
			Average:	33	20-45

* The mutation rates of these strains were also measured using the 1000-compartment fluctuation test.

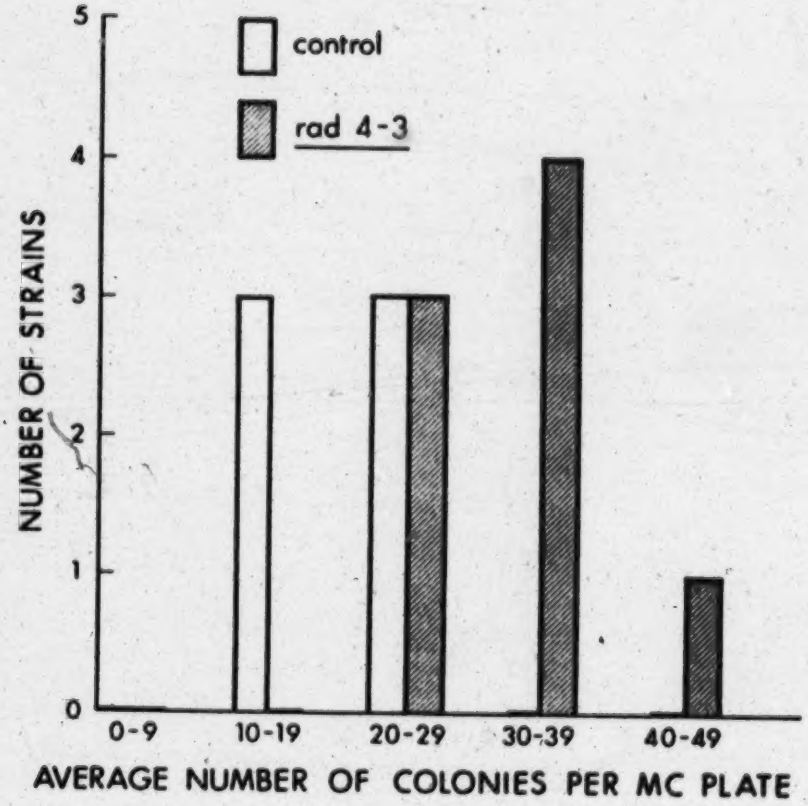


Figure 28. Frequency distributions of the average number of colonies per MC plate in the UV-sensitive and UV-insensitive strains derived from the cross of S960-1A rad4-3 x XV185-14C.

Tables 14 and 15 give the "lassie" test data for the RAD5-1 and rad5-1 strains respectively. The frequency distributions of the average numbers of colonies per MC plate are shown in Figure 29. The rad5-1 allele-bearing strains show an obviously increased MC colony count of approximately six to seven times. Averages for both UV-sensitive and UV-insensitive strains correlate well with observed reversion rates. There is a good deal of variation in the average colony count observed in rad5-1 strains. Some strains give less than 100 revertant colonies per MC plate whereas others show more than 300. A similar variation, though not as great, is observed in the actual mutation rates.

The unusual situation presented by the rad8-1 allele is illustrated in Table 16 and Figure 30. UV-sensitive strains give an average of 10 colonies per MC plate, in agreement with their average reversion rate of 1.1×10^{-8} mutational events per cell per generation. Of the UV-insensitive strains derived from the rad8-1 cross, most indicate, by the number of revertants growing on MC plates, their average reversion rate of 3.4×10^{-8} mutational events per cell per generation. However, the UV-insensitive strain XV420-7A tends to show a suspiciously lower than usual colony count on MC.

Table 17 shows no significant difference between rad10-1 and the RAD10-1 strains on the basis of colony counts on MC. Both the reversion rates of UV-sensitive and UV-insensitive strains observed agree with those predicted on the basis of the "lassie" data. Once again the higher reversion rate observed for XV365-2A is manifested in a higher "lassie" count. The frequency distribution is found in

TABLE 14. Number of colonies on MC plates shown by the UV-insensitive haploid strains derived from the cross of S228-6B rad5-1 x XV185-14C

Strain	UV Sensitivity	Average No. of Background Revertants	No. of Plates	Average No. of Colonies Per MC Plate	Range
*XV419-11A	Insensitive	0	3	22	20-25
*XV419-12A		1	3	29	20-40
XV419-14A		1	5	30	20-40
XV419-15A		0	3	27	20-40
XV419-16A		1	3	28	25-35
XV419-21A		2	2	35	25-45
XV419-22A		0	1	19	20
XV419-23A		0	2	42	40-45
XV419-29A		1	2	47	40-55
XV419-30A		12	2	53	45-60
XV419-32A		0	0	27	25-30
XV419-33A		2	2	22	15-25
XV419-36A		0	2	27	25-30
XV419-37A		0	2	27	20-30
XV419-40A		27	2	74	70-80
XV419-42A		6	2	36	35
XV419-46A		0	2	38	35-40
XV419-47A		0	2	16	15-20
XV419-48A		1	2	28	20-35
XV419-53A		0	2	19	15-20
XV419-54A		44	2	62	60-65
XV419-56A		0	2	18	15-20
XV419-57A		0	2	27	20-30
XV419-58A		0	2	21	15-25
XV419-59A		85	2	112	80-140
XV419-60A		0	1	33	35
XV419-61A		0	2	18	15-20
XV419-62A		0	2	28	25-35
XV419-63A		0	2	14	15
XV419-66A		0	2	37	30-40
			Average:	28	15-45

* The mutation rates of these strains were also measured using the 1000-compartment fluctuation test.

TABLE 15. Number of colonies on MC plates shown by the UV-sensitive haploid strains derived from the cross of S228-6B rad5-1 x XV185-14C

Strain	UV Sensitivity	Average No. of Background Revertants	No. of Plates	Average No. of Colonies Per MC Plate	Range
*XV419-2A	Sensitive	2	5	65	55-85
XV419-3A		5	3	157	135-175
XV419-4A		1	4	78	65-90
*XV419-5A		4	3	250	225-285
XV419-8A		34	3	254	205-315
XV419-10A		5	5	72	50-120
*XV419-13A		2	5	147	125-175
XV419-17A		67	2	316	275-355
XV419-18A		3	2	338	295-385
XV419-20A		16	1	287	285
XV419-24A		29	2	116	110-120
XV419-26A		39	2	321	290-355
XV419-27A		20	2	348	345-350
XV419-28A		228	2	489	455-525
XV419-34A		5	2	156	130-180
XV419-35A		20	2	283	265-300
XV419-38A		2	2	386	365-410
XV419-39A		5	2	112	110-115
XV419-41A		11	2	139	135-140
XV419-44A		32	1	245	245
XV419-45A		103	2	384	370-395
XV419-49A		1	2	160	160
XV419-50A		56	2	161	155-165
XV419-51A		1	2	189	175-205
XV419-52A		5	2	221	185-255
XV419-64A		0	2	132	120-145
XV419-65A		1	2	174	170-180
XV419-68A		19	2	116	90-140
			Average:	191	65-390

* The mutation rates of these strains were also measured using the 1000-compartment fluctuation test.

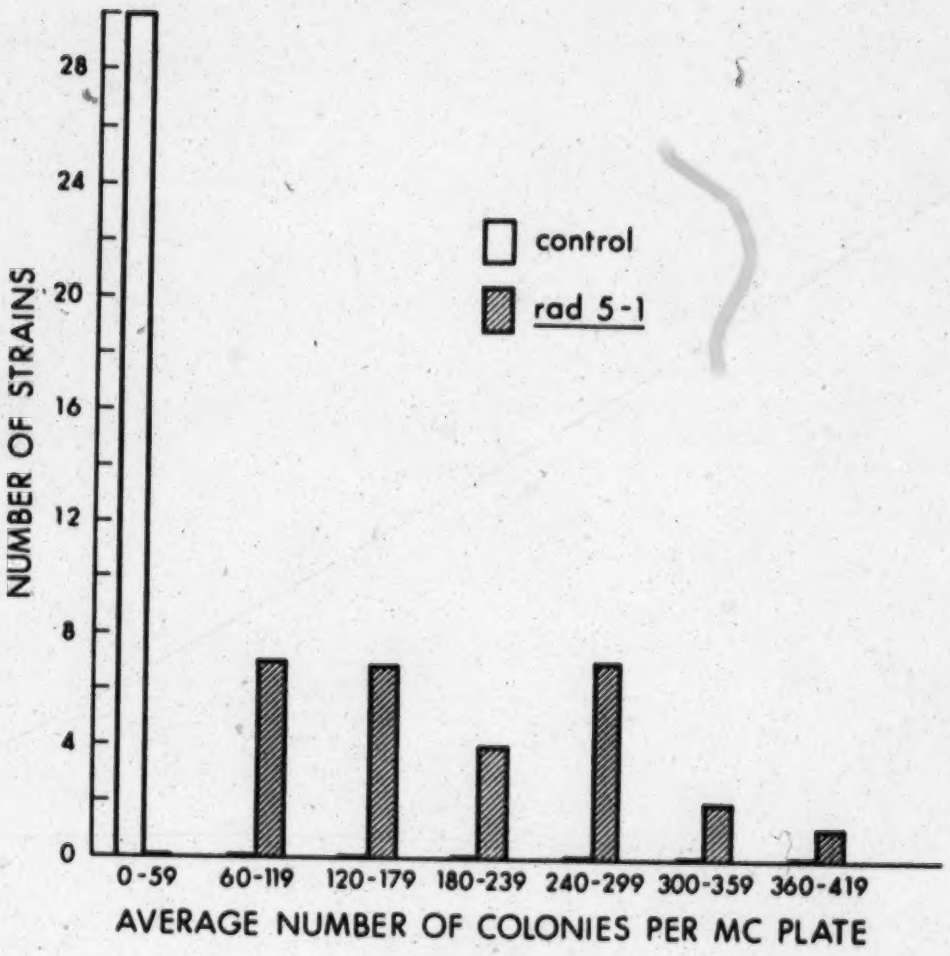


Figure 29. Frequency distributions of the average number of colonies per MC plate in the UV-sensitive and UV-insensitive strains derived from the cross of S228-6B rad5-1 x XV185-14C.

TABLE 16. Number of colonies on MC plates shown by the UV-sensitive and UV-insensitive haploid strains derived from the cross of 197/2D rad8-1 x XV185-14C

Strain	UV Sensitivity	Average No. of Background Revertants	No. of Plates	Average No. of Colonies Per MC Plate	Range
*XV420-4A	Insensitive	5	3	48	45-50
XV420-5A		0	3	36	30-50
*XV420-6A		0	3	25	20-30
XV420-7A		1	5	10	10-15
XV420-8A		4	3	38	35-40
			Average:	31	10-50
*XV420-1A	Sensitive	0	3	13	5-20
*XV420-2A		2	3	15	10-20
*XV420-3A		0	3	3	0-5
			Average:	10	5-15

* The mutation rates of these strains were also measured using the 1000-compartment fluctuation test.

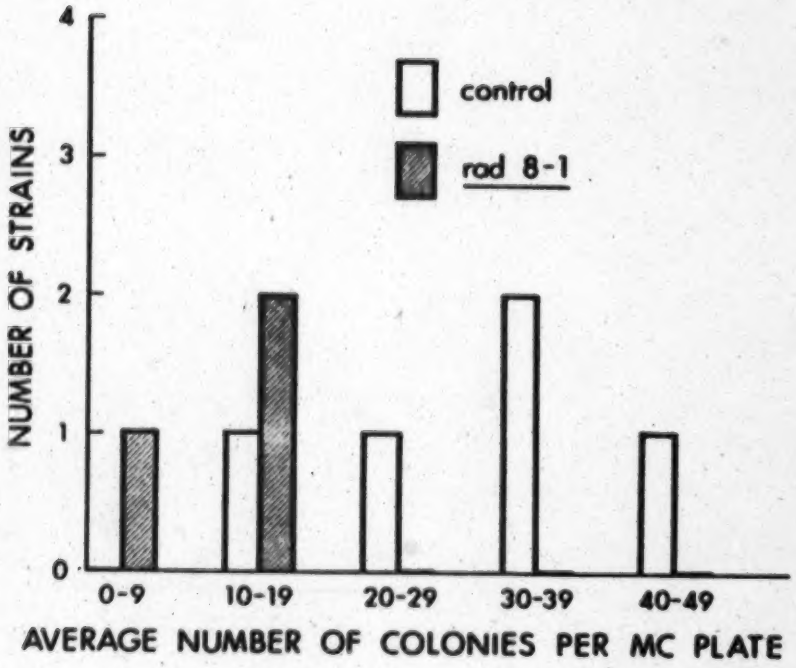


Figure 30. Frequency distributions of the average number of colonies per MC plate in the UV-sensitive and UV-insensitive strains derived from the cross of 197/2D rad8-1 x XV185-14C.

TABLE 17. Number of colonies on MC plates shown by the UV-sensitive and UV-insensitive haploid strains derived from the crosses of S296-3C rad10-1 x XV169-15A and XV365-3A rad10-1 x XV169-15A

Strain	UV Sensitivity	Average No. of Background Revertants	No. of Plates	Average No. of Colonies Per MC Plate	Range
XV366-1A	Insensitive	0	12	17	10-25
*XV366-2A		0	8	30	20-40
*XV366-4A		0	11	16	10-20
*XV366-5A		1	6	22	10-25
XV366-8A		1	10	23	20-25
			Average:	22	15-30
*XV365-2A	Sensitive	0	14	49	35-65
*XV365-4A		0	12	34	20-45
*XV366-3A		2	7	28	20-40
*XV366-6A		1	10	17	10-30
XV366-7A		1	5	24	15-35
			Average:	30	15-50

* The mutation rates of these strains were also measured using the 1000-compartment fluctuation test.

Figure 31.

The strain bearing the rad18-2 allele gave 68 and 101 colonies on each of two MC plates, with a negligible number of background revertants. This again qualitatively indicates a mutator though the actual rate cannot be predicted accurately from the "lassie" test.

A most peculiar situation exists in the strains bearing the rad18-1 allele, as is evident by the variation observed in the spontaneous mutation rates. "Lassie" test data (Table 18 and Figure 32) indicate that most of the UV-insensitive strains show a very low revertant count on MC and the revertant colony size is usually very small or tiny. Only one UV-insensitive strain (XV362-2A) shows a normal colony size and count and thus, if its lysine reversion rate were accurately measured, it would probably be at normal UV-insensitive strain values. Of the UV-sensitive strains only two show normal colony size while the two others tested give poor "lassies" with decreased colony size. It is thus apparent that some other segregating factor(s) is(are) responsible for these effects. The possibility also exists that many of these strains are slow growing because the number of revertants in box experiments with XV362-5A had not yet reached a true plateau level after 14 days and was still increasing.

In order to examine this situation further, the strain XV362-5A, carrying the rad18-1 allele and showing a slightly higher than normal mutation rate was crossed with XV185-14C, the standard control strain. Daughter strains obtained from this cross were examined by means of the "lassie" test. MC plates were scored as usual after 10 days' incubation and colony counts recorded, but the plates were returned

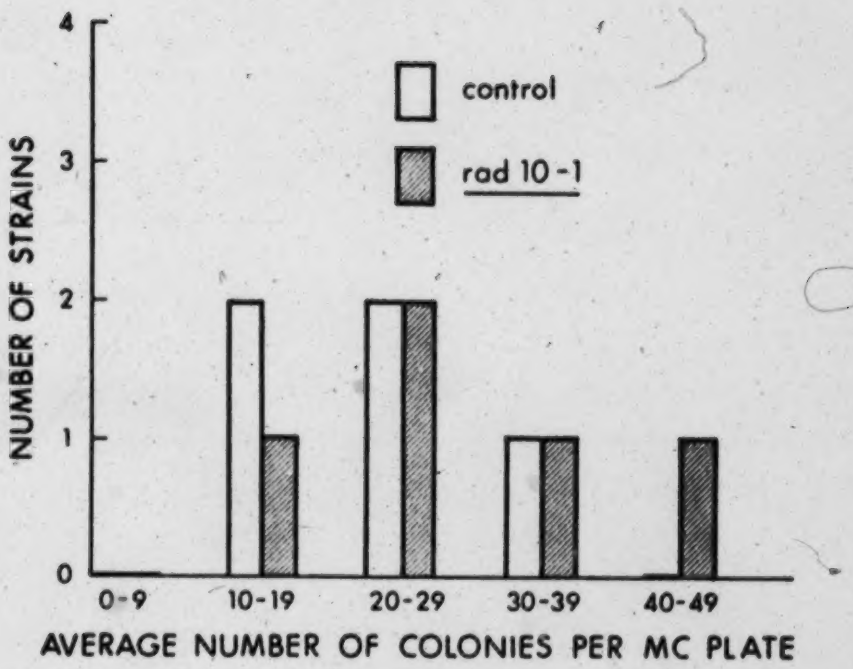


Figure 31. Frequency distributions of the average number of colonies per MC plate in the UV-sensitive and UV-insensitive strains derived from the crosses of S962-3C rad10-1 x XV169-15A and XV365-3A rad10-1 x XV169-15A.

TABLE 18. Number of colonies on MC plates shown by the UV-sensitive and UV-insensitive haploid strains derived from the cross of JCG133/3A rad18-1 x XV169-15A

Strain	UV Sensitivity	Average No. of Background Revertants	No. of Plates	Average No. of Colonies Per MC Plate	Range	Colony Size
XV362-1A	Insensitive	0	6	3	0-5	S
XV362-2A		0	9	25	20-35	N
*XV362-3A		0	8	4	0-5	S
XV362-4A		0	9	7	5-10	S
XV362-10A		0	7	2	0-5	T
XV362-11A		0	7	11	5-15	S
XV362-12A		0	7	3	0-10	T
XV362-13A		0	6	2	0-5	S
XV362-16A		0	6	11	0-20	T
XV362-22A		0	3	3	0-10	S
*XV362-5A	Sensitive	1	2	49	50	N
*XV362-6A		0	6	1	1	S
XV362-8A		0	6	29	20-35	N
XV362-20A		0	2	12	10-15	S

* The mutation rates of these strains were also measured using the 1000-compartment fluctuation test.

N = Normal colony size.

S = Smaller than normal colony size.

T = Tiny colonies.

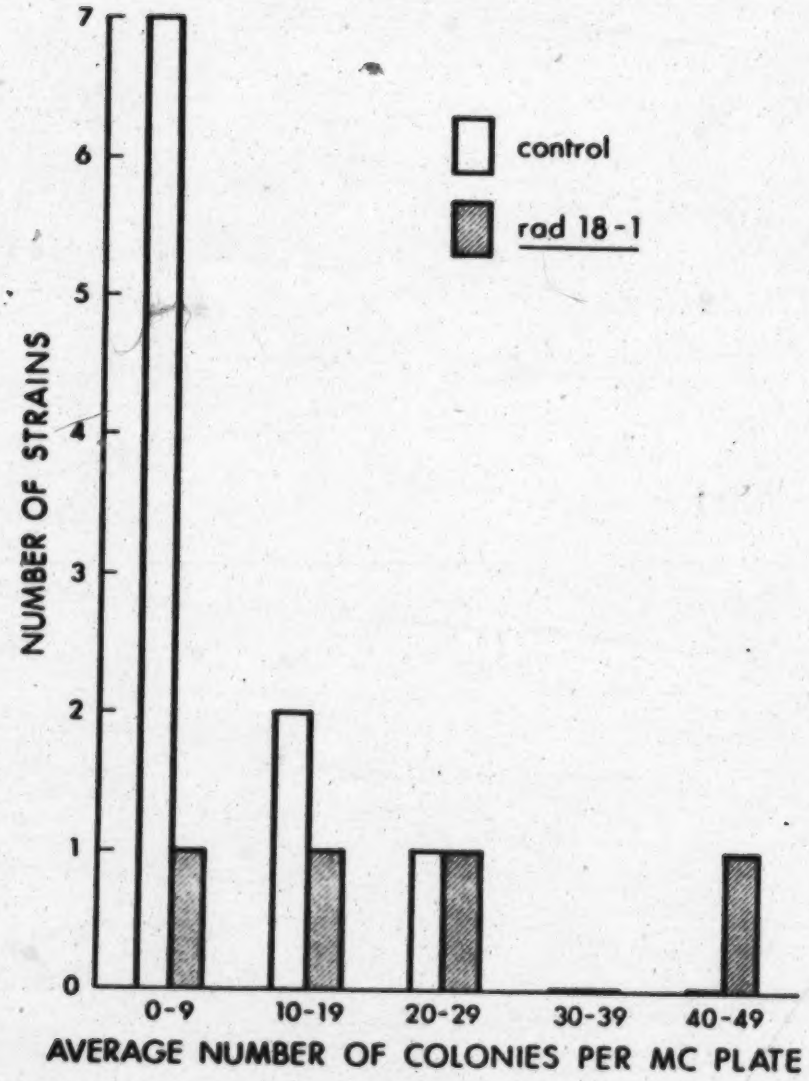


Figure 32. Frequency distributions of the average number of colonies per MC plate in the UV-sensitive and UV-insensitive strains derived from the cross of JCG133/3A rad18-1 x XV169-15A.

to the incubator in plastic bags and left to incubate for approximately two and a half more weeks. A final colony count after one month's incubation was then made. The results are shown in Tables 19 and 20. The RAD18-1 strains can be classified into three groups. One group gives a normal non-mutator control colony count on MC with normal-sized colonies. The second and largest group consists of slow-growing strains: even after one month of incubation, only an average of 10 colonies per plate are counted. A third group comprises very tiny colonies which do not increase in size or change in number after one month. The UV-sensitive strains also fall into three classes: those with normal colony size and an average MC colony count of 43 with little or no change in 10 to 30 days' incubation; those which give a poor "lassie" with small-sized colonies at 10 days but which show an approximately average (but not mutator) revertant count of normal colony size after one month; and a few which give a poor "lassie" even after one month's incubation. It seems as if the first group represents strains which grow at a normal rate, are (apparently) unaffected by any segregating factor(s), and show an average or higher (approximately 50) colony count on MC. The second group seems to be slow-growing in that it gives a normal "lassie" if given sufficient time to grow, yet the colony count is only at UV-insensitive strain values. The third group seems to remain the same even after one month's growth. The corresponding frequency distrubutions are given in Figures 33 and 34.

TABLE 19. Number of colonies on MC plates shown by UV-insensitive haploid strains derived from the cross XV362-5A rad18-1 x XV185-14C

Strain	Avg. No. of Background Revertants	No. of Plates	Avg. No. of Colonies Per MC Plate*	Range*
XV424-22A	1	4	21(21)	10-20
XV424-23A	1	4	21(30)	10(25)-30
XV424-24A	0	4	15(23)	5(10)-30
XV424-25A	2	3	16(16)	10-25
XV424-27A	1	3	30(35)	20(30)-45(50)
XV424-32A	1	4	17(25)	5(10)-55
XV424-36A	0	2	16(16)	10-20
XV424-51A	0	2	19(22)	15-25
XV424-54A	0	2	16(19)	15-20
		Average[1]	19(23)	
XV424-26A	0	4	8(10)	0(5)-10(15)
XV424-28A	0	4	6(12)	5(10)-(15)
XV424-29A	0	4	5(12)	0(5)-10(20)
XV424-30A	0	4	3(8)	0(5)-5(10)
XV424-31A	0	4	3(8)	0(5)-5(15)
XV424-33A	0	4	3(7)	0(5)-5(10)
XV424-34A	0	4	2(8)	0(5)-5(10)
XV424-35A	0	4	4(14)	0(10)-10(17)
XV424-37A	0	4	8(15)	0(10)-10(20)
XV424-38A	0	2	9(14)	5(10)-10(15)
XV424-39A	0	2	2(11)	0-5(10)
XV424-40A	0	2	3(9)	0-5(10)
XV424-41A	1	2	4(10)	0-5(10)
XV424-42A	1	2	10(11)	10(10)-(15)
XV424-43A	0	2	4(7)	0-5(5)
XV424-44A	0	2	12(15)	10-15(15)
XV424-45A	0	2	10(14)	10(10)-(15)
XV424-47A	0	2	6(15)	5(15)
XV424-48A	0	2	6(14)	5(10)-10(15)
XV424-49A	1	2	6(14)	5(15)
XV424-50A	0	2	8(15)	5-15(15)
XV424-52A	1	2	11(15)	5(10)-20
XV424-53A	0	2	5(10)	5(10)
XV424-55A	0	2	8(12)	5(10)-10(15)
XV424-56A	0	2	7(8)	5(10)
XV424-57A	1	2	2(11)	0-5(11)
XV424-58A	0	2	3(8)	0(5)-5(10)
XV424-59A	0	2	7(11)	5(10)-10(15)
XV424-60A	0	2	1(2)	0-5
		Average[2]	6(11)	
XV424-18A	0	4	7	0-10
XV424-19A	0	4	12	10-20
XV424-20A	1	3	5	5-10
XV424-21A	0	4	7	5-10
		Average[3]	8	

* The first number indicates colony count after 10 days' incubation; the number in brackets indicates colony count after one month's incubation.

1 = Normal size colony.

2 = Small and normal-sized colonies after 10 days; normal colony size after one month.

3 = Very tiny colonies even after one month.

TABLE 20. Number of colonies on MC plates shown by the UV-sensitive haploid strains derived from the cross XV362-5A rad18-1 x XV185-14C

Strain	Avg. No. of Background Revertants	No. of Plates	Avg. No. of Colonies Per MC Plate*	Range*
XV424-2A	1	3	76(76)	65-80
XV424-5A	0	4	31(31)	20-45
XV424-6A	0	4	45(46)	40-55
XV424-7A	4	4	39(46)	25-55(70)
XV424-8A	0	3	68(69)	60-75
XV424-9A	1	3	55(57)	50-60
XV424-11A	1	4	46(52)	35(45)-60
XV424-12A	1	4	24(28)	10-35
XV424-13A	1	4	20(27)	15-35(40)
XV424-61A	0	2	66(66)	65-70
XV424-62A	1	2	25(25)	20-25
XV424-63A	1	2	50(50)	50
XV424-65A	1	2	18(37)	15(30)-20(40)
		Average[1]	43(47)	

Strain	Avg. No. of Background Revertants	No. of Plates	Avg. No. of Colonies Per MC Plate*	Range*
XV424-1A	0	4	8(11)	0-15(20)
XV424-3A	1	4	10(25)	5-15(40)
XV424-4A	1	4	11(28)	5(20)-15(45)
XV424-10A	0	3	2(30)	0-5(30)
XV424-14A	0	4	12(30)	0(15)-30(50)
XV424-15A	0	4	1(11)	0(10)-5(15)
XV424-16A	10	4	27(44)	20(30)-40(55)
		Average[2]	10(26)	
XV424-17A	0	4	1(1)	0-5
XV424-64A	0	2	1(2)	0-5
XV424-66A	0	2	1(7)	1(5)-(10)
XV424-67A	0	2	1(11)	1(10)-(15)
		Average[3]	1(5)	

* The first number indicates colony count after 10 days' incubation; number in brackets indicates colony count after one month's incubation.

1 = Normal colony size.

2 = Small colonies after 10 days' incubation but normal colony size after one month.

3 = Small colony size persists even after one month.

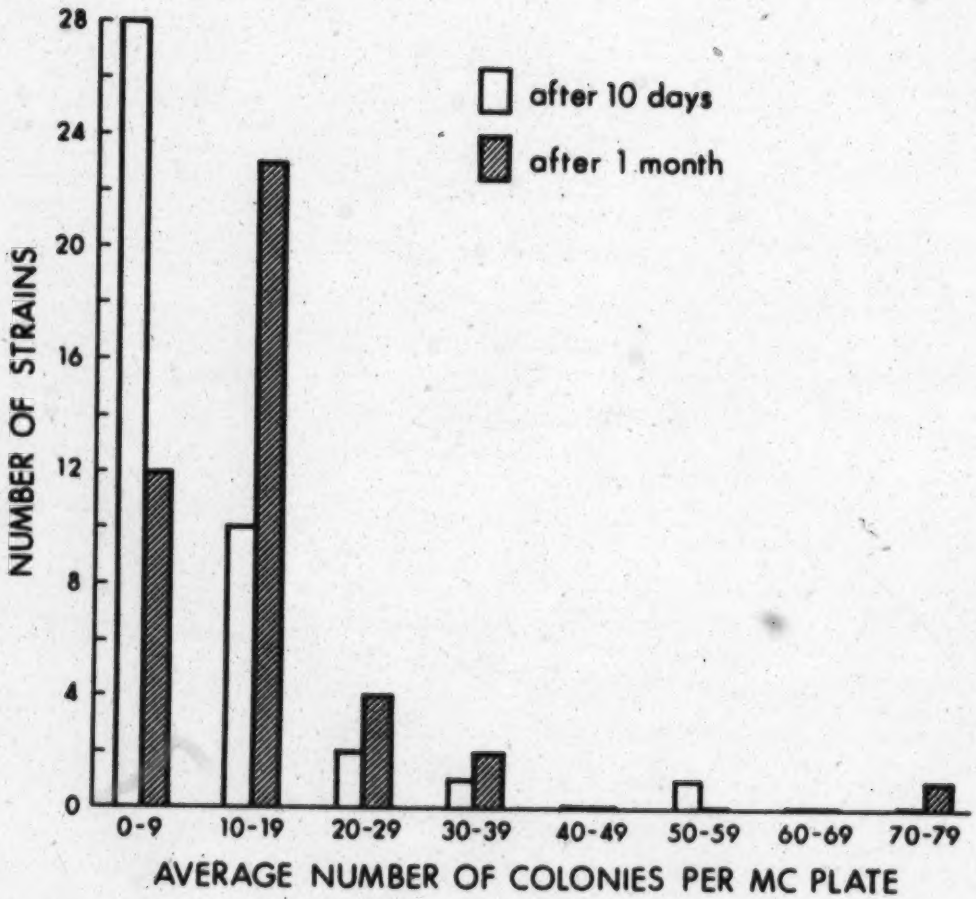


Figure 33. Frequency distributions of the average number of colonies per MC plate in the UV-insensitive strains derived from the cross of XV362-5A rad18-1 x XV185-14C.

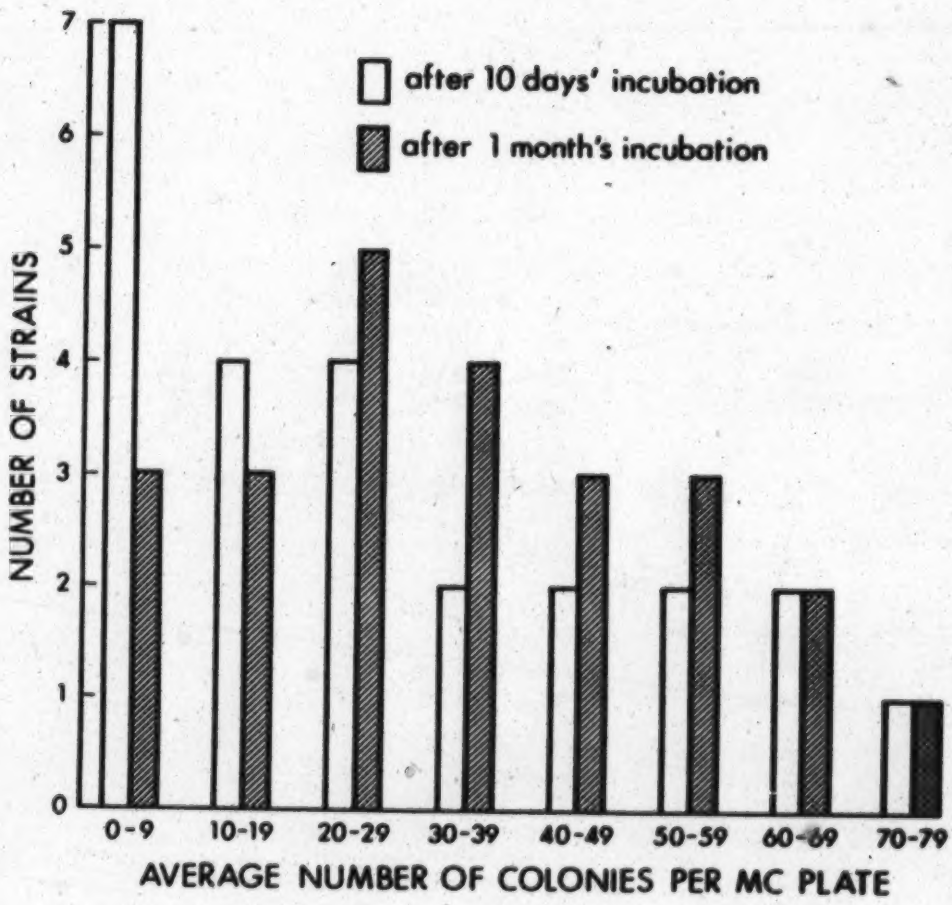


Figure 34. Frequency distributions of the average number of colonies per MC plate in the UV-sensitive strains derived from the cross of XV362-5A rad18-1 x XV185-14C.

DISCUSSION

A summary of the most important results obtained in these experiments, as well as other pertinent information, is presented in Table 21.

In this study, the spontaneous reversion rates to lysine and histidine independence were investigated in several strains bearing radiosensitivity alleles. The lys1-1 allele is supersuppressible (Hawthorne and Mortimer, 1963) and is an ochre nonsense mutant (Hawthorne, 1969), showing the characteristic properties of no intragenic complementation, no osmotic remediability, and no temperature sensitivity (Manney, 1964; Hawthorne and Friis, 1964; Fink, 1966). It can be reverted either by a reversion at the lys1 locus itself, possibly through a transversion (UAA→UAC) (von Borstel et al., 1973), or by a forward mutation at a supersuppressor gene locus. The former situation results only in lysine independence while the latter simultaneously abolishes several other nonsense mutants included in the genotype of the strain (Hawthorne and Mortimer, 1963). Twenty-two supersuppressors are known to map throughout the yeast genome (Gilmore et al., 1971) and are classified as to the nonsense alleles they suppress and their efficiency of suppression (Hawthorne and Mortimer, 1963; Gilmore, 1967). The seven Class I supersuppressors suppress the alleles trp5-48, arg4-17, his5-2, lys1-1, and ade2-1 (Gilmore, 1966; Gilmore, 1967; Hawthorne and Mortimer, 1968) and are believed to be genes encoding tyrosine tRNAs (Gilmore, 1967; Gilmore et al.,

TABLE 21. Summary of information related to the rad alleles investigated

rad Allele	UV-Sensitivity	Repair Pathway Affected	Average Mutation Rate (Total Lysine)	Average Mutation Rate (Histidine)	Comments
RAD (Avg.)	-	-	2.7	8.4	-
rad1-1	SSS	Excision	4.0	14.7	non-mutator
rad2-2	SS	Excision	2.6	11.4	non-mutator
rad3-12	S	Excision	13.9	107.2	mutator
rad4-3	SS	Excision	2.3	13.2	non-mutator
rad5-1	S	Post-replication?	16.7	26.0	mutator
rad8-1	S	?	1.1	3.0	antimutator? (modifiers)
rad10-1	SS	?	2.6	14.4	non-mutator
rad18-1	SS	Post-replication?	?	?	weak mutator? (modifiers)
rad18-2	SS	Post-replication?	13.8	-	mutator

SSS = Highly UV-sensitive; SS = UV-sensitive; S = Weakly UV-sensitive.

1968; Gilmore et al., 1971). Suppression is believed to occur through a forward mutation (transition, transversion, or addition) in the anticodon of the tyrosine tRNA or possibly by a modification outside the anticodon (Gilmore et al., 1971; von Borstel, 1969b), which consequently allows the tyrosine tRNA to recognize a nonsense codon and insert tyrosine at that position in the protein (Gilmore, 1967; Gilmore et al., 1968). In the experiments reported here, the lys1-1 supersuppressor revertants were mostly Class I suppressors, as verified by the pattern of suppression of the nonsense alleles carried by the strains. However, a few suppressors show the pattern of suppression classified as suppressor 11 (Class II Set 1). These are distinguishable from Class I suppressors because they do not suppress ade2-1 (Gilmore, 1966). Suppressor 11 also is believed to insert tyrosine in response to a nonsense codon (Gilmore et al., 1971).

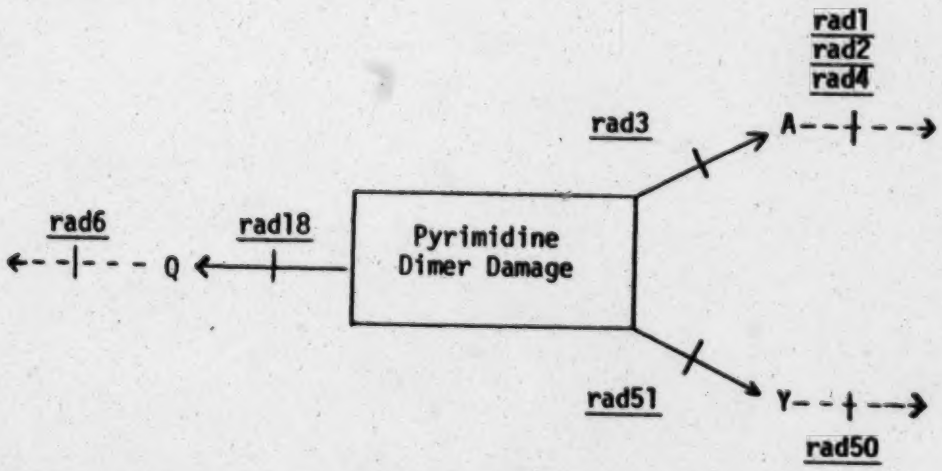
The his1-7 allele, on the other hand, is thought to be a missense mutant, because it is not suppressible by an ochre suppressor, shows allelic complementation, is osmotically remedial, and reverts to feedback resistance (Hawthorne and Friis, 1964; Korch and Snow, 1973). Because of the above properties and because of its high spontaneous reversion rate and the heterogeneity of the revertant colonies, it is believed to revert most commonly through internal missense suppression. The possibility of the occurrence of some external suppressors at low frequency, however, has not been eliminated. A reversion to histidine independence does not result in the loss of other nutritional requirements, so any external suppression would have to be something other than ochre-nonsense suppression.

With reference to Table 21, it is apparent that only those strains bearing the radiosensitivity alleles rad3-12, rad5-1, or rad18-2 show a significant increase in spontaneous mutation rate to lysine or histidine independence. The rad3-12 and rad5-1 alleles both increase the lysine reversion rate through supersuppressor mutation as well as the histidine reversion rate, and rad3-12 apparently affects an increase in lysine locus reversions also. In agreement with the finding of mutator activity in the rad5-1 allele-bearing strains, rev2-1 (rad5-5), allelic to rad5-1, is also a spontaneous mutator (Lemontt, 1972; 1973). The rad18-2 mutant was here only tested for lysine reversion but earlier work (von Borstel et al., 1971) showed that it increased the mutation rate to both lysine and uracil independence. The specificity of these mutators, though, cannot be accurately predicted because of the various mechanisms by which supersuppressor mutations can occur (von Borstel et al., 1973). However, it has been suggested that rad18-2 is an addition-deletion mutator (von Borstel et al., 1971), because the ura4-11 mutant shows a meiotic effect (Magni, 1963; 1969).

The increase in mutation rate observed in strains carrying these alleles is supported by the "lassie" test data. As all the UV-sensitive strains tested carrying the rad5-1 allele consistently showed a very high colony count on MC, indicative of mutator activity, while all the UV-insensitive strains showed average non-mutator values, it seems very likely that the mutator activity and UV-sensitivity found in the rad5-1 allele-bearing strains is due to a mutation in the same gene, or else, if these effects are caused by mutations in

two different genes, these two genes must be very closely linked (within 2 centimorgans). A similar conclusion might be applicable to rad3 and rad18, on the basis of the available data, but it should be noted that the number of strains tested, by means of the "lassie" test, was not as great as for rad5-1. The contradictory data observed for rad18-1 need not necessarily conflict with that observed for rad18-2, as will be discussed below.

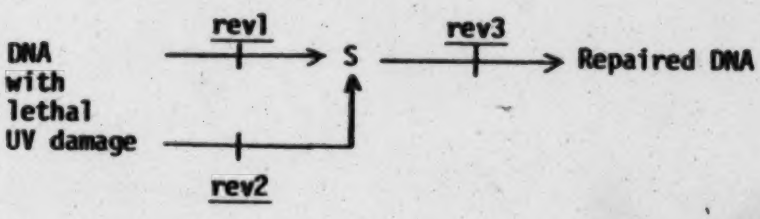
An interesting point to note with respect to these three loci is that they each mediate the first step in one of the pathways leading to repair of UV-induced pyrimidine dimer damage, according to the scheme of Cox presented below (Game and Cox, 1973; Cox and Game, 1974). Dashed lines indicate an unknown number of steps in the reaction sequence.



The gene product of RAD3 is believed to mediate the first step in the excision-repair pathway (Game, 1971; Game and Cox, 1972), while RAD18 may control the first step in a damage-non-specific pathway leading perhaps to a recombinational type of repair, possibly post-replication repair (Brendel and Haynes, 1973), though experiments to show its

involvement in post-replication repair are inconclusive (Cox and Game, 1974). The RAD51 gene product is required for the first step of a third repair pathway not involved in post-replication repair (Cox and Game, 1974).

The position of rad5 on this scheme of pathways for repair of pyrimidine dimer damage can only be speculated upon. One of the rev genes (Lemontt, 1971a), namely rev2-1, is an allele of this locus (rad5-5). The following pathway has been envisualized for REV-controlled repair (Lemontt, 1970; 1971b):



Because rev mutations (rev1 and rev3) decrease the UV-induced mutability of the strains carrying them, it might be that the wild type REV genes control error-prone pathways which repair secondary DNA lesions, such as daughter strand gaps, and, consequently, generate UV-induced mutations (Lemontt, 1971a and b; Lemontt, 1972). The relation of the pathway controlled by RAD5 to that of excision repair is unclear. As double mutant interaction studies have not been done with rad5 and rad3 mutants, one cannot definitely conclude whether the RAD5-controlled reaction sequence branches off from the intermediate produced by the RAD3-controlled step of excision repair (Game, 1971), or whether RAD5 is involved in another pathway completely separated from that of excision repair.

As the exact nature of spontaneous premutational lesions in the DNA is unknown, it is not clear if they can actually be repaired by some or all of the same mechanisms which effect dark repair of UV-induced DNA damage. The possibility that some premutational spontaneous damage may be similar to dimer type damage still exists. From the data presented above, it seems that the RAD5 gene product might be required for the normal repair of spontaneous premutational lesions. In other words, a pathway (in which one step, perhaps the initial one, is controlled by the RAD5 gene) may repair such lesions, or, at least, one step of this pathway (that mediated by RAD5) might be required to channel the lesions to repair. If radiation-sensitivity and mutator activity in rad18 and rad3 mutants are also due to a single mutation in the RAD18 gene and the RAD3 gene, respectively, then their wild type products might also be required to convert premutational damage into a form which can be acted upon by other repair enzymes.

If, for the moment, one assumes that each of the above three loci do indeed mediate the first steps in three different repair pathways and also that spontaneous lesions are at least partially repaired by the same mechanisms as UV-induced lesions, then it would seem that, when the first intermediate in a pathway is not made due to a mutation in the gene encoding the enzyme responsible for the first step, the damaged DNA cannot be adequately repaired via that pathway. If the different repair pathways repair different types of spontaneous lesions, and a mutation at the first step of a pathway yields accumulation of unrepaired damage which is mutagenic, then a

mutation at any of these rad loci would increase spontaneous mutability. An explanation, more in keeping with that postulated for UV-induced damage repair, may be that a mutation blocking the first step in a repair pathway leads to the damage being repaired via another pathway. For example, in the case of the rad3 mutant, the premutational lesion cannot be repaired by excision (if it can be repaired by excision at all) and also would not be repairable through an escape pathway controlled by RAD5 and branching from the excision repair pathway. If it is to be repaired, the damage may be channelled to repair via an independent RAD5 pathway (that is, one not forming a branch of the excision pathway) if the damage is accessible to the action of that enzyme. Likewise, the RAD18-mediated pathway may be a likely candidate for alternative repair, as it seems to repair both X-ray and UV-induced damage (Resnick, 1969; Brendel and Haynes, 1973). If RAD18 repairs through a post-replication repair process, which is error-prone, it would lead to an increase in spontaneous mutability. A similar explanation for the rad18 mutant would involve postulating repair through an independent RAD5-mediated pathway, if such a pathway exists, or through the RAD3 and then RAD5 branch pathway. In either case repair might produce mutations if these pathways are error-prone. If the damage is repairable by excision, this might indicate that excision repair is not error-free in yeast, provided that most of the damage load, blocked to repair through RAD18, goes through the excision repair pathway and not another error-prone pathway. A mutation at rad5 once again would leave open the error-prone RAD18 pathway or else excision repair if it is capable of repairing the damage.

Within the context of repair pathways, one may also speculate about the existence of, as yet, undiscovered pathway(s) not involved in repair of UV damage but which can repair spontaneous lesions and, if blocked, lead to increased mutability. Perhaps some of the rad mutations are involved in such pathways also. Still another explanation, assuming similar repair mechanisms for UV- and spontaneously-induced lesions, may be that, since the mutators rad5-1 and rad3-12 are relatively less UV-sensitive than other rad alleles tested, perhaps they possess some residual repair, as has been suggested for the most resistant rad1 and rad3 (including rad3-12) mutants (Parry et al., 1972; Waters and Parry, 1973). Perhaps the slow error-prone repair of some lesions by means of a partly defective enzyme may occur to increase not only the survival of strains bearing these alleles but also to increase their mutability.

The high spontaneous mutability observed in rad3 strains may also be explained if rad3 is postulated to encode a nuclease which cannot recognize the mutational lesions normally removed in wild type RAD3 strains. Possibly rad3 may encode a defective endonculease, a plausible speculation as RAD3 is thought to control the first step in excision repair. It has been suggested that spontaneous mutation rates may reflect the relative rates of nucleotide insertion and removal during DNA synthesis and mutations which decrease the amount of nuclease in relation to polymerase may produce mutators (Muzycka et al., 1972).

In contrast to the rad3 mutant discussed above, mutations at the other three loci involved in excision repair, namely rad1, rad2,

and rad4, do not show any appreciable increase in spontaneous mutability to lysine or histidine independence. The variation observed in the rad1-1 strain is evident also in "lassie" test data and part of it may reflect differences in the genetic background of the strains. Because both UV-sensitive and UV-insensitive strains show this variation, because the increase (for lysine reversion rates at least) is not outside the normally observed non-mutator control strain values, and because the increase is not uniformly observed (for histidine reversion rates), it is concluded that rad1-1 has no significant effect on spontaneous mutability to lysine and histidine independence. The increase in histidine reversion rate observed for rad4-3 allele-bearing strains is probably not significant. Although kinetic curves for revertant appearance show no distinct plateau, the increase in revertants after 10 days is not very great.

It is, however, interesting to compare the spontaneous mutability found for these loci by other investigators. Moustacchi (1969) found an increased spontaneous reversion rate for many auxotrophic markers in strains bearing the rad1-3 allele. Zakharov et al., (1970) also found increased spontaneous reversion to adenine independence in rad2-18-bearing strains, while von Borstel et al., (1971) showed rad2-16 and rad2-17 to have no enhancing effect on lysine reversion rate. In attempting to interpret the variability of results, one should remember first of all that the reversion rates in each case were measured using different systems. For example, only lys1-1 and his1-7 reversion rates were examined in this study, as compared to reversion for different mutant alleles studied by other researchers. The reversion rate to

adenine independence, using Zakharov's adenine-requiring mutant for instance, was not measured for rad2-2 strains. Thus, one cannot, on the basis of work here with rad1-1 and rad2-2, conclude infallibly that these radiosensitive mutants do not increase the spontaneous mutation rate in general, because possibly they may increase reversion rates in different systems which may represent different types of lesions. Also, different rad alleles at one locus represent mutations at different points within that gene and perhaps a mutation at only a certain point(s) increases mutability. As was mentioned earlier, the rad1-1 allele displayed a variable mutation rate ranging from low to high non-mutator values, within the normal range observed for UV-insensitive strains. If rad1-1 is a mutator, though, it is at best a very weak one. Therefore, the observed variation with rad1-1 was not considered significant. Lastly, it can be stated that, although the method used for measuring mutation rate in this study is quite accurate, many variables are known which exert weak effects on spontaneous mutation rates.

The rad10-1 mutant may also, within limits, be concluded to have no effect on reversion rate to histidine and lysine independence. As RAD10-1 has not been positioned in any pathway to date, its role in repair must remain a mystery for the present.

At first glance the rad8-1 mutant seems to be an antimutator, especially as it decreases the lysine supersuppressor mutation rate as compared to the UV-insensitive strains. However, one of these insensitive strains also displays a very low rate of reversion to histidine independence. Thus, it cannot be concluded that the UV-

sensitivity and antimutator effects are associated. It appears more likely that an antimutator in the genetic background may be segregating. Further investigation is necessary in order to understand the role in controlling spontaneous mutation rates for this rad allele, whose role in repair is also as yet undefined.

In the above discussion it was concluded that the rad18-2 mutation increases reversion rates to lysine and uracil independence. The results obtained with rad18-1 may at first seem contradictory to this hypothesis. However, lassie test data obtained from a second cross, involving a rad18 strain with slightly greater than wild type mutation rate, indicated varying degrees of a depressing effect on the spontaneous mutability of some strains, both UV-sensitive and UV-insensitive. Meanwhile, some UV-sensitive strains seemed to indicate a slight increase in mutability. Evidently, rad18-1 is not associated with antimutator activity but rather an unlinked modifier or modifiers is(are) affecting the results obtained and seem to confer an antimutator effect on supersuppressor mutations. These modifiers seem also to affect growth rate, colony size, and mutation rate. It seems likely that rad18-1 has weak mutator activity but this mutator property is overshadowed by the modifier(s) and is not expressed in some UV-sensitive strains. Thus, rad18-1 itself also merits further investigation, but only after it has been incorporated into a modifier-free background.

In conclusion, it has been shown that rad3-12, rad5-1, and rad18-2 increase the spontaneous reversion rate to lysine independence and rad3-12 and rad5-1 increase it to histidine independence. The

other *rad* alleles tested showed either no effect or require further investigation. Although the question of the association of UV-sensitivity and spontaneous mutability must still remain unanswered, the possibility remains open that at least some genes controlling radiation-sensitivity may affect spontaneous mutability and control the repair of spontaneous as well as UV-induced damage.

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APPENDIX

TABLE A1. Survival after ultraviolet irradiation of haploid strains bearing the rad alleles involved in the excision repair and related pathways

Strain	Time of Irradiation (min)	Dilution Plated	Colony Count	Surviving Fraction
X1687-12B	0	-4	105	
	1	-4	92	0.88
	3	-3	151	0.14
	5	-2	264	0.025
XS774-5D rad1-1	0	-4	213	
	1	-1	3	0.000014
	3	-0	3	0.0000014
	5	-0	4	0.0000018
S226-7C rad2-2	0	-4	192	
	1	-1	307	0.0016
	3	-0	13	0.0000067
	5	-0	14	0.0000072
197/2D rad3-12	0	-4	234	
	1	-4	42	0.18
	3	-2	19	0.00081
	5	-1	6	0.000026
S960-1A rad4-3	0	-4	149	
	1	-1	218	0.0015
	3	-0	43	0.000029
	5	-0	13	0.0000087
S228-6B rad5-1	0	-4	177	
	1	-4	83	0.47
	3	-2	64	0.0036
	5	-1	11	0.000062
KC376 rad52-1	0	-4	210	
	1	-4	129	0.61
	3	-2	560	0.027
	5	-1	185	0.00088

TABLE A2. Survival after ultraviolet irradiation of haploid strains bearing the rad alleles involved in other unknown repair pathways

Strain	Time of Irradiation (min)	Dilution Plated	Colony Count	Surviving Fraction
X1687-12B	0	-4	105	
	1	-4	92	0.88
	3	-3	151	0.14
	5	-2	264	0.025
197/2D rad8-1	0	-4	153	
	1	-4	38	0.25
	3	-2	233	0.015
	5	-1	135	0.00088
S962-3C rad10-1	0	-4	156	
	1	-1	1548	0.0099
	3	-0	328	0.00021
	5	-0	22	0.000014
JCG133/3A rad18-1	0	-4	167	
	1	-1	16	0.000096
	3	-0	14	0.0000083
	5	-0	5	0.0000029
KC372 rad18-2	0	-4	77	
	1	-1	94	0.0012
	3	-0	42	0.000055
	5	-0	6	0.0000077
KC376 rad52-1	0	-4	210	
	1	-4	129	0.61
	3	-2	560	0.027
	5	-1	185	0.00088

TABLE A3. Survival after ultraviolet irradiation of haploid strains bearing the rad1-1 allele

Strain	Time of Irradiation (min)	Dilution Plated	Colony Count	Surviving Fraction
X1687-12B	0	-4	161	
	1	-4	121	0.75
	3	-3	119	0.074
	5	-2	159	0.0099
KC376 rad52-1	0	-4	85	
	1	-4	42	0.49
	3	-2	120	0.014
	5	-1	13	0.00015
XV363-1A rad1-1	0	-4	139	
	1	-1	15	0.00011
	3	-0	5	0.0000035
	5	-0	11	0.0000079
XV363-2A rad1-1	0	-4	109	
	1	-1	7	0.000064
	3	-0	12	0.000011
	5	-0	6	0.0000055
XS774-5D rad1-1	0	-4	213	
	1	-1	3	0.000014
	3	-0	3	0.0000014
	5	-0	4	0.0000018

TABLE A4. Survival after ultraviolet irradiation of haploid strains bearing the rad2-2 allele

Strain	Time of Irradiation (min)	Dilution Plated	Colony Count	Surviving Fraction
X1687-12B	0	-4	194	
	1	-4	162	0.84
	3	-3	166	0.086
	5	-2	177	0.0091
KC376 rad52-1	0	-4	139	
	1	-4	70	0.50
	3	-2	232	0.017
	5	-1	72	0.00052
XV361-1A rad2-2	0	-4	116	
	1	-1	416	0.0036
	3	-0	53	0.000046
	5	-0	18	0.000016
S226-7C rad2-2	0	-4	192	
	1	-1	307	0.0016
	3	-0	13	0.0000067
	5	-0	14	0.0000072

TABLE A5. Survival after ultraviolet irradiation of haploid strains bearing the rad10-1 allele and derived from the diploid XV365

Strain	Time of Irradiation (min)	Dilution Plated	Colony Count	Surviving Fraction
X1687-12B	0	-4	78	
	1	-4	66	0.85
	3	-3	210	0.27
	5	-2	402	0.05
KC376 rad52-1	0	-4	47	
	1	-4	27	0.57
	3	-2	180	0.038
	5	-1	92	0.002
XV365-2A rad10-1	0	-4	194	
	1	-1	2040	0.011
	3	-0	410	0.00021
	5	-0	49	0.000025
XV365-3A rad10-1	0	-4	126	
	1	-1	1280	0.01
	3	-0	317	0.00025
	5	-0	24	0.000019
XV365-4A rad10-1	0	-4	109	
	1	-1	3200	0.029
	3	-0	1920	0.0018
	5	-0	809	0.00074
S962-3C rad10-1	0	-4	156	
	1	-1	1548	0.0099
	3	-0	328	0.00021
	5	-0	22	0.000014

TABLE A6. Survival after ultraviolet irradiation of haploid strains bearing the rad10-1 or its wild type allele and derived from the diploid XV366

Strain	Time of Irradiation (min)	Dilution Plated	Colony Count	Surviving Fraction
X1687-12B	0	-4	73	
	1	-4	68	0.93
	3	-3	179	0.25
	5	-2	192	0.026
XV366-2A	0	-4	173	
	1	-4	120	0.69
	3	-3	265	0.15
	5	-2	353	0.02
XV366-3A rad10-1	0	-4	219	
	1	-1	1940	0.0089
	3	-0	545	0.00025
	5	-0	8	0.0000037
XV366-4A	0	-4	201	
	1	-4	150	0.75
	3	-3	380	0.19
	5	-2	399	0.02
XV366-5A	0	-4	130	
	1	-4	114	0.88
	3	-3	300	0.23
	5	-2	658	0.051
XV366-6A rad10-1	0	-4	166	
	1	-1	1980	0.012
	3	-0	710	0.00043
	5	-0	54	0.000033
S962-3C rad10-1	0	-4	156	
	1	-1	1548	0.0099
	3	-0	328	0.00021
	5	-0	22	0.000014

TABLE A7. Survival after ultraviolet irradiation of haploid strains bearing the rad18-1 or its wild type allele

Strain	Time of Irradiation (min)	Dilution Plated	Colony Count	Surviving Fraction
X1687-12B	0	-4	194	
	1	-4	162	0.84
	3	-3	166	0.086
	5	-2	177	0.0091
KC376 rad52-1	0	-4	139	
	1	-4	70	0.50
	3	-2	232	0.017
	5	-1	72	0.00052
XV362-3A	0	-4	118	
	1	-4	98	0.83
	3	-3	426	0.36
	5	-2	673	0.057
XV362-5A rad18-1	0	-4	114	
	1	-1	57	0.0005
	3	-0	76	0.000067
	5	-0	29	0.000025
XV362-6A rad18-1	0	-4	150	
	1	-1	52	0.00035
	3	-0	18	0.000012
	5	-0	7	0.0000046
JCG133/3A rad18-1	0	-4	167	
	1	-1	16	0.000096
	3	-0	14	0.0000083
	5	-0	5	0.0000029

TABLE A8. Colony counts on MC in UV-sensitive and UV-insensitive haploid strains derived from the crosses of XS774-5D *rad1-1* x XV169-12A and XS774-5D *rad1-1* x XV185-6A

Strain	No. of Background Revertants (per -LYS plate)		No. of Colonies per MC plate
XV363-11A	0	1	27 34
XV421-2B	0	1	68
XV421-3C	0	0	78
XV421-5D	0	0	24 35 46
XV421-7D	0	0	33 41
XV421-9D	0	1	17 17 26
XV421-13D	0	1	24 31 40
XV421-15B	0	1	16 28
XV421-16C	0	0	27 29
XV421-18B	0	0	22 24
XV363-1A	0	2	29 53
XV363-1A	5	11	43 44 44 51 53
XV363-1A	0	0	28 34 34 35 38 39 45 56
XV363-2A	12	14	47 51
XV363-2A	0	0	36 38 40 43 46 49 52
XV363-2A	0	1	35 37 40 41 48
XV363-10A	0	0	34 43
XV421-4A	0	0	32 39
XV421-4C	0	0	37 62
XV421-5A	0	1	26 29
XV421-6A	0	2	40 55
XV421-8C	1	2	57 76
XV421-12A	0	1	30 55 63
XV421-17A	18	22	31 35 52

TABLE A9. Colony counts on MC in UV-sensitive and UV-insensitive haploid strains derived from the crosses of S226-7C rad2-2 x XV169-15A and S226-7C rad2-2 x XV185-14C

Strain	No. of Background Revertants (per -LYS plate)		No. of Colonies per MC plate									
XV361-2A	0	1	15	19	21	22	23	25	26	28	32	35
XV422-3A	0	0	19	20								
XV422-4A	0	1	31	36	43							
XV422-6A	0	0	20	26	30							
XV422-9A	7	8	35	38	39							
XV422-10A	0	1	15	18	24							
XV422-11A	0	0	14	17								
XV422-15A	0	0	17	22								
XV361-1A	0	0	17	21								
XV422-1A	0	0	21	25								
XV422-2A	3	11	32	43								
XV422-5A	0	0	28	34	49							
XV422-8A	0	0	14	21	22							
XV422-12A	0	0	32	34								
XV422-13A	1	3	32	64								
XV422-14A	0	0	34	45								

TABLE A10. Colony counts on MC in UV-sensitive and UV-insensitive haploid strains derived from the cross of 197/2D rad3-12 x XV185-14C

Strain	No. of Background Revertants (per -LYS plate)		No. of Colonies per MC plate							
XV423-3A	0	0	31	49						
XV423-4A	0	0	17	18						
XV423-9A	1	1	35	39						
XV423-10A	0	0	18	21						
XV423-11A	0	1	17	23						
XV423-12A	0	0	36	37						
XV423-13A	0	0	12	13						
XV423-14A	0	0	11	16	19	20	20	20	24	27	32
XV423-18A	1	3	47	57						
XV423-20A	0	1	45	58						
XV423-21A	0	0	30	43						
XV423-22A	5	7	29	35						
XV423-25A	0	0	15	21						
XV423-26A	3	6	33	42						
XV423-1A	0	2	89	92						
XV423-2A	0	0	54	83						
XV423-5A	0	1	159	165						
XV423-6A	0	0	71	93						
XV423-7A	2	2	44	64	67	76	81			

TABLE A11. Colony counts on MC in UV-sensitive and UV-insensitive haploid strains derived from the cross of S960-1A rad4-3 x XV185-14C

Strain	No. of Background Revertants (per -LYS plate)		No. of Colonies per MC plate		
XV418-8A	0	1	10	11	15
XV418-9A	0	0	9	12	13
XV418-3B	1	1	25	31	32
XV418-5B	0	0	14	18	20
XV418-6B	0	1	20	30	31
XV418-7B	7	9	21	30	37
XV418-1A	0	5	20	29	35
XV418-2A	0	0	27	34	35
XV418-3A	16	20	45	52	68
XV418-4A	0	2	26	39	44
XV418-5A	43	62	78	92	103
XV418-6A	0	1	23	25	27
XV418-7A	1	2	40	43	55
XV418-2B	0	0	12	26	28

TABLE A12. Colony counts on MC in UV-insensitive haploid strains derived from the cross of S228-6B rad5-1 x XV185-14C

Strain	No. of Background Revertants (per -LYS plate)		No. of Colonies per MC plate				
XV419-11A	0	0	19	22	27		
XV419-12A	0	1	19	28	40		
XV419-14A	0	1	19	30	32	35	38
XV419-15A	0	0	19	26	38		
XV419-16A	0	1	23	29	33		
XV419-21A	1	2	26	44			
XV419-22A	0	0	19				
XV419-23A	0		38	45			
XV419-29A	0	1	38	56			
XV419-30A	10	13	46	60			
XV419-32A	0		24	29			
XV419-33A	1	3	16	27			
XV419-36A	0	0	25	29			
XV419-37A	0	0	22	32			
XV419-40A	27		69	78			
XV419-42A	1	10	35	36			
XV419-46A	0	0	37	39			
XV419-47A	0	0	13	19			
XV419-48A	0	1	21	35			
XV419-53A	0	0	15	22			
XV419-54A	28	61	58	65			
XV419-56A	0	0	17	19			
XV419-57A	0	0	21	32			
XV419-58A	0	0	17	24			
XV419-59A	75	95	81	142			
XV419-60A	0	0	33				
XV419-61A	0	0	14	22			
XV419-62A	0	0	23	23			
XV419-63A	0	0	14	14			
XV419-66A	0	0	32	41			

TABLE A13. Colony counts on MC in UV-sensitive haploid strains derived from the cross of S228-6B rad5-1 x XV185-14C

Strain	No. of Background Revertants (per -LYS plate)		No. of Colonies per MC plate				
XV419-2A	1	3	54	60	64	68	83
XV419-3A	1	8	137	162	174		
XV419-4A	0	1	66	73	84	91	
XV419-5A	3	5	223	244	283		
XV419-8A	32	35	204	244	314		
XV419-10A	3	7	49	54	68	70	119
XV419-13A	1	2	124	136	151	153	174
XV419-17A	67		276	356			
XV419-18A	1	6	294	383			
XV419-20A	13	18	287				
XV419-24A	28	29	110	122			
XV419-26A	39	39	288	355			
XV419-27A	18	22	345	352			
XV419-28A	185	280	454	524			
XV419-34A	4	7	131	181			
XV419-35A	20		267	299			
XV419-38A	1	2	363	408			
XV419-39A	5	6	108	116			
XV419-41A	11	12	136	142			
XV419-44A	32		245				
XV419-45A	95	111	372	395			
XV419-49A	0	1	158	161			
XV419-50A	54	57	155	167			
XV419-51A	0	1	173	203			
XV419-52A	4	6	186	256			
XV419-64A	0		120	145			
XV419-65A	0	1	170	178			
XV419-68A	15	23	92	140			

TABLE A14. Colony counts on MC in UV-sensitive and UV-insensitive haploid strains derived from the cross 197/2D rad8-1 x XV185-14C

Strain	No. of Background Revertants (per -LYS plate)		No. of Colonies per MC plate				
XV420-4A	3	6	45	48	51		
XV420-5A	0	0	29	31	48		
XV420-6A	0	0	20	25	32		
XV420-7A	1	2	6	7	8	15	15
XV420-8A	3	5	35	36	42		
XV420-1A	0	0	7	11	20		
XV420-2A	1	2	12	14	20		
XV420-3A	0	0	3	3	4		

TABLE A15. Colony counts on MC in UV-sensitive and UV-insensitive haploid strains derived from the crosses of S962-3C rad10-1 x XV169-15A and XV365-3A x XV169-15A

Strain	No. of Background Revertants (per -LYS plate)		No. of Colonies per MC plate													
XV366-1A	0	0	8	11	13	15	16	16	18	20	20	20	24	24		
XV366-2A	0	0	21	23	24	24	28	37	38	41						
XV366-4A	0	0	9	13	15	15	17	17	17	18	18	19	20			
XV366-5A	1	1	12	21	21	24	24	27								
XV366-8A	0	1	18	20	20	20	21	24	25	25	26	26				
XV365-2A	0	0	36	41	42	43	44	49	50	50	51	52	53	56	63	65
XV365-4A	0	0	21	26	27	28	29	34	37	39	40	42	44	46		
XV366-3A	2	3	18	25	25	31	31	33	38							
XV366-6A	0	1	12	13	16	17	18	19	19	20	28	29				
XV366-7A	0	1	15	23	24	25	34									

TABLE A16. Colony counts on MC in UV-sensitive and UV-insensitive haploid strains derived from the cross of JCG133/3A rad18-1 x XV169-15A

Strain	No. of Background Revertants (per -LYS plate)		No. of Colonies per MC plate									Majority Colony Size
XV362-1A	0	0	0	2	2	3	4	5				S
XV362-2A	0	0	20	23	25	25	27	31	32	33	35	N
XV362-3A	0	0	1	3	3	4	5	6	6	6		S
XV362-4A	0	0	4	5	5	5	7	7	10	10	11	S
XV362-10A	0	0	0	1	1	1	2	3	4			T
XV362-11A	0	0	6	9	10	14	13	13	15			S
XV362-12A	0	0	0	0	0	1	1	1	8			T
XV362-13A	0	0	0	1	2	2	3	4				S
XV362-16A	0	0	0	0	8	14	20	22				T
XV362-22A	0	0	0	3	7							S
XV362-5A	0	2	48	50								N
XV362-6A	0	0	1	1	1	1	1	1				S
XV362-8A	0	0	18	21	30	34	35	37				N
XV362-20A	0	0	10	13								S

N = Normal size.
S = Smaller than normal colony size.
T = Tiny colonies.

TABLE A17. Colony counts on MC in UV-insensitive haploid strains derived from the crosses of XV362-5A rad18-1 x XV185-14C

Strain	No. of Background Revertants (per -LYS plate)		No. of Colonies Per MC Plate*				Strain	No. of Background Revertants (per -LYS plate)		No. of Colonies Per MC Plate*			
XV424-22A	0	1	11(11)	14(14)	15(15)	22(22)	XV424-42A	0	1	8(9)	12(13)		
XV424-23A	1	1	10(30)	21(26)	24(32)	30(32)	XV424-43A	0	0	2(7)	2(6)		
XV424-24A	0	0	6(9)	10(19)	16(34)	29(32)	XV424-44A	0	0	11(13)	13(16)		
XV424-25A	0	3	9(11)	15(15)	23(23)		XV424-45A	0	0	9(13)	11(14)		
XV424-27A	0	1	22(28)	27(30)	43(48)		XV424-47A	0	0	6(15)	6(14)		
XV424-32A	0	1	3(10)	4(21)	5(13)	57(57)	XV424-48A	0	0	5(14)	7(13)		
XV424-36A	0	1	10(10)	22(22)			XV424-49A	0	1	4(14)	7(14)		
XV424-51A	0	0	14(17)	24(27)			XV424-50A	0	0	3(13)	13(17)		
XV424-54A	0	0	17(21)	15(16)			XV424-52A	0	1	5(9)	18(22)		
							XV424-53A	0	0	4(10)	6(9)		
XV424-26A	0	0	2(4)	6(10)	10(11)	12(17)	XV424-54A	0	0	17(21)	15(16)		
XV424-28A	0	0	4(11)	4(15)	7(9)	7(13)	XV424-55A	0	0	7(13)	8(11)		
XV424-29A	0	0	2(10)	2(6)	5(15)	9(19)	XV424-56A	0	0	6(7)	8(9)		
XV424-30A	0	0	1(6)	4(12)	4(9)	4(4)	XV424-57A	0	1	1(7)	2(15)		
XV424-31A	0	0	1(5)	2(5)	2(7)	5(15)	XV424-58A	0	0	2(6)	3(11)		
XV424-34A	0	0	0(10)	1(4)	1(8)	6(10)	XV424-59A	0	0	7(13)	7(8)		
XV424-35A	0	0	1(13)	2(15)	5(10)	8(17)	XV424-60A	0	0	1(1)	1(2)		
XV424-37A	0	0	1(11)	8(16)	11(14)	11(19)							
XV424-38A	0	0	6(12)	12(16)			XV424-18A	0	0	5	6(11)	13	4(10)
XV424-39A	0	0	2(9)	2(12)			XV424-19A	0	0	1(18)	5(11)	7(8)	8(13)
XV424-40A	0	0	1(9)	4(10)			XV424-20A	0	1	0(5)	1(3)	3(9)	
XV424-41A	0	1	3(11)	5(9)			XV424-21A	0	0	0(6)	5(8)	5(10)	6(6)

* The first number indicates colony count after 10 days' incubation; the number in brackets indicates colony count after one month's incubation.

TABLE A18. Colony counts on MC in UV-sensitive haploid strains derived from the cross XV362-5A rad18-1 x XV185-14C

Strain	No. of Background Revertants (per -LYS plate)		No. of Colonies Per MC Plate*				Strain	No. of Background (Revertants (per -LYS plate)		No. of Colonies Per MC Plate*			
XV424-2A	0	1	64(64)	71(71)	79(79)		XV424-1A	0	0	0(0)	2(12)	9(12)	13(20)
XV424-5A	0	0	22(22)	26(26)	30(30)	45(45)	XV424-3A	0	1	5(5)	8(25)	12(32)	17(40)
XV424-6A	0	0	38(38)	40(40)	47(50)	53(57)	XV424-4A	0	0	7(25)	10(18)	12(46)	17(24)
XV424-7A	4	5	25(25)	29(35)	50(52)	55(71)	XV424-10A	0	0	1(32)	2(28)	2(32)	
XV424-8A	0	0	61(63)	69(69)	75(75)		XV424-14A	0	0	1(14)	4(12)	17(42)	28(52)
XV424-9A	0	1	49(51)	57(60)	60(61)		XV424-15A	0	0	0(15)	1(10)	1(10)	4(9)
XV424-11A	0	1	33(47)	41(52)	49(49)	62(62)	XV424-16A	8	11	20(32)	20(46)	28(43)	41(56)
XV424-12A	1	2	10(10)	24(28)	27(36)	35(37)							
XV424-13A	1	1	14(14)	14(21)	20(35)	33(39)							
XV424-61A	0	0	64(64)	68(68)			XV424-17A	0	0	0	1	1	2
XV424-62A	1	1	22(22)	27(27)			XV424-64A	0	0	0(3)	1(1)		
XV424-63A	0	1	49(49)	51(51)			XV424-66A	0	0	1(5)	1(10)		
XV424-65A	0	1	14(32)	22(42)			XV424-67A	0	0	1(11)	1(12)		

* The first number indicates colony count after 10 days' incubation; the number in brackets indicates colony count after one month's incubation.